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(54) A colorectal cancer suppressor gene related protein

(57) [Abstract]
[PROBLEM TO BE SOLVED] To discover a novel substance that is associated with the colorectal tumor suppressor gene APC in order to enable control of APC and to control colorectal tumors.
[SOLUTION] A novel protein M1 having an ability to bind to the APC gene product, particularly to the armadillo repeat (Arm) domain of the gene product, which is namely a polypeptide consisting of the amino acid sequence set forth in SEQ ID No: 1 of the Sequence Listing, a polypeptide containing part of the polypeptide, a polynucleotide encoding the polypeptide or its complementary strand, a vector containing the polynucleotide or its complementary strand, a transformant containing the vector, an antibody against the polypeptide, a method for producing the polypeptide, a method for screening inhibitors, an antagonist and activator of the function and expression of M1 using the aforementioned products, and a compound identified by the method are provided, and further a pharmaceutical composition and diagnostic means for use in colorectal tumors are provided using the aforementioned products.

[0009] Furthermore, the novel protein M1 was found to have, as a function thereof, GEF activity specific to Rac, a member of the Rho family. Namely, the novel protein M1 binds to Rac to promote GDP/GTP exchange reaction and activates Rac, and further acts on NF κB, c-jun, SRE, etc. located downstream of cell signaling in which Rac is involved. Besides, the novel protein M1 may induce cell lamellipodia and membrane ruffling that result from the physiological function of Rac, and thus its involvement in cell adhesion is presumed.

[0010] Regarding the localization of the APC gene product within a cell, it is reported that the APC gene product accumulates at the ends of microtubules in the migrating cell when the cell migrates from the crypt to the tip of intestinal villus (J. Cell Biol., 134:165-179, 1996). The novel protein M1 of the present invention has been found to accumulate also in similar areas within the cell. These facts raise the possibility that the novel protein M1 of the present invention holds a key to the regulation of cell migration in intestinal villus.

[0027] (Screening) The novel protein M1 thus prepared and the polypeptide derived therefrom, the polynucleotide encoding thereof and its complementary strand, the cell transformed based on the information about the amino acid sequences and base sequences thereof, and the antibody that immunologically recognizes the novel protein M1 or the polypeptide derived therefrom provide, when using them solely or in combination, an effective means to screen for an inhibitor or activator that acts on the binding of the novel protein M1 and the polypeptide derived therefrom to the APC gene product, the function of

the novel protein M1 including its GEF activity, or the expression of the novel protein M1. Namely, it is possible to provide a screening method to obtain a compound that inhibits or enhances the binding of the polypeptide of the present invention to the APC gene product utilizing at least either of the polypeptide or the antibody of the present invention; a screening method for a compound that reacts with the polynucleotide of the present invention and inhibits or enhances the expression of the polynucleotide utilizing at least any one of the polynucleotide, the vector, the tranformant, and the antibody of the present invention; and a screening method for a compound that inhibits or enhances the function of the polypeptide of the present invention including the GEF activity utilizing at least either of the polypeptide and the antibody of the present invention. For example, the screening of an antagonist by the drug design based on the stereo-structure of the polypeptide, the screening of an expression-regulating agent by gene level using a protein-synthesizing system, and the screening of an antibody-recognizing substance using an antibody, can be carried out by using one of well-known drug-screening systems.

[0029] The candidate compounds thus selected can be used in preparing a pharmaceutical composition for use in the treatment of colorectal tumors by selecting ones by taking a balance between their biological usefulness and toxicity into consideration. Besides, the novel protein M1 and the polypeptide derived therefrom, the polynucleotide encoding thereof and its complementary strand, the vector containing the nucleotide sequence thereof, and the antibody that immunologically recognizes the novel protein M1 and the polypeptide derived therefrom can be used themselves as a pharmaceutical means for use in treating colorectal tumors which have a function to inhibit, to antagonize, or to activate the interaction of the novel protein M1 with the APC gene product. Here, colorectal tumors include both benign and malignant tumors, specifically exemplified by familial adenomatous polyposis (FAP) and colon cancer. For the pharmaceutical preparation, it is sufficient to use a well-known means suitable for preparing each of the objects such as peptide, protein, polynucleotide, or antibody.

[0044] (Localization of M1 within a cell) Next, the novel protein M1 of the present invention was studied for its localization within a cell. First, a Madin-Darby canine kidney (MDCK) epithelial cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). cDNAs of full-length M1 and M1ΔNB (amino acids 127-619) were subcloned into pcDNA 3.1(+) that is a mammalian expression vector having a CMV promotor. Plasmid DNA, pMKITneo-Myc-tagged APC-arm, was constructed by inserting a DNA fragment encoding a partial sequence of APC (amino acids 446-880) into pMKITneo having a SRα promotor. The expression plasmid was transfected into MDCK cells by using LipofectAMINE (Life Technologies Co.) according to the instruction manual.

[0045] MDCK cells were fixed with 3.7% formaldehyde in phosphate buffered saline (PBS) at 4°C for one hour. The fixed cells were treated with Tris-buffered saline (TBS) containing 0.2% triton X-100 at room temperature for 10 minutes and washed three times with TBS.

After permeabilization, the cells were incubated with a primary antibody in TBS containing 1% BSA, 3% FBS and 0.2% triton X-100 at room temperature for one hour. After removing the primary antibody, the cells were washed three times with TBS. Bound primary antibody was detected by using a goat secondary antibody (Cappel Co.) labeled with FITC (fluorescein isothiocyanate) or rhodamine. The stained samples were examined under a Carl Zeiss LSM510 Laser scanning microscope.

[0046] The results are shown in Fig. 9. The cell shown in Fig. 9a is the same as that shown in Fig. 9b, which was made to express HA-tagged M1 protein. The cell shown in Fig. 9c is the same as that shown in Fig. 9d, which was made to express both HA-tagged M1 protein and Myc-tagged APC-arm. The cell shown in Fig. 9e is a cell that was made to express HA-tagged M1ΔNB (amino acids 127-619). The cell shown in Fig. 9f is a cell that was made to express Myc-tagged APC-arm. The cell shown in Fig. 9a and Fig. 9b was double-stained with an anti-HA antibody and an anti-APC antibody. The cell shown in Fig. 9c and Fig. 9d was doubled stained with an anti-HA antibody and an anti-Myc antibody. The cell shown in Fig. 9e was treated with an anti-HA antibody. The cell shown in Fig. 9f was treated with an anti-Myc antibody. As indicated by arrowheads in Fig. 9b, clusters of APC protein were present in membrane extensions. Furthermore, as indicated by arrows in Fig. 9a ~ d, M1 and APC proved to be co-localized at the same area of the epithelial cell periphery.

[0047] (Morphological analysis of M1 expressing cell line) Furthermore, an MDCK cell line stably expressing M1ΔNB was established to examine the effect of M1 on cell morphology and cytoskeleton. Five clones of the M1ΔNB expressing cell line all showed a tendency to grow dispersedly without closely aggregating to each other. The novel protein M1 was presumed to be involved in cell adhesion.

[0048]
[EFFECT OF THE INVENTION] As explained above, M1 of the present invention is a novel protein that characteristically binds to the armadillo repeat domain of the APC gene product and possesses GEF (guanine nucleotide exchange factor) activity. A novel pharmaceutical composition and a diagnostic means that use such characteristics above will provide great usefulness in the clinical and basic medical field relating to the APC gene product.

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(54)【発明の名称】　大腸癌抑制遺伝子関連蛋白質

(57)【要約】
【課題】大腸癌抑制遺伝子ＡＰＣに関与する新規物質を見いだし、ＡＰＣの制御を可能にし、大腸癌を制御すること。
【解決手段】ＡＰＣ遺伝子産物、特に該遺伝子産物のアルマジロリピート（Ａｒｍ）部位との結合能をもつ新規蛋白質Ｍ１、すなわち配列表の配列番号１に示すアミノ酸配列からなるポリペプチド、及び該ポリペプチドの一部を有するポリペプチド、該ポリペプチドをコードするポリヌクレオチドまたはその相補鎖、該ポリヌクレオチドまたはその相補鎖を含むベクター、該ベクターを有する形質転換体、当該ポリペプチドに対する抗体、当該ポリペプチドの製造法、上記のものを利用したＭ１の機能および発現の阻害剤・拮抗剤・賦活剤のスクリーニング方法、該方法で同定された化合物を提供し、更にこれらを利用し、大腸腫瘍に用いる医薬組成物・診断手段を提供する。

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【特許請求の範囲】

【請求項１】　下記の群より選ばれるポリペプチド；

①配列表の配列番号１に記載のアミノ酸配列で示されるポリペプチド、

②前記①のポリペプチドのアミノ酸配列を含有するポリペプチド、

③前記①のポリペプチドと少なくとも約７０%のアミノ酸配列上の相同性を有しかつ大腸癌の癌抑制遺伝子（Ａｄｅｎｏｍａｔｏｕｓ　Ｐｏｌｙｐｏｓｉｓ　Ｃｏｌｉ：ＡＰＣ）の遺伝子産物のアルマジロリピート部位をコードするポリペプチドに対する結合能を有するポリペプチド、および

④前記①から③のポリペプチドのアミノ酸配列において１ないし数個のアミノ酸の欠失、置換、付加などの変異あるいは誘発変異を有し、かつＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有するポリペプチド。

【請求項２】　配列表の配列番号１に記載のアミノ酸配列の少なくとも５個のアミノ酸配列を有し、かつＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有するポリペプチド。

【請求項３】　請求項１または２に記載のポリペプチドをコードするポリヌクレオチドまたはその相補鎖。

【請求項４】　請求項３に記載のポリヌクレオチドまたはその相補鎖とストリンジェントな条件下でハイブリダイゼーションするポリヌクレオチド。

【請求項５】　配列表の配列番号２に記載のポリヌクレオチドまたはその相補鎖の塩基配列のうち少なくとも１５個の連続した塩基配列で示されるポリヌクレオチドであって、該ポリヌクレオチドの転写によって発現されるポリペプチドがＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有する、ポリヌクレオチド。

【請求項６】　請求項３から５のいずれか１項に記載のポリヌクレオチドを含有する組換えベクター。

【請求項７】　請求項６の組換えベクターで形質転換された形質転換体。

【請求項８】　請求項７の形質転換体を培養する工程を含む、請求項１または２に記載のポリペプチドの製造方法。

【請求項９】　請求項１または２に記載のポリペプチドを免疫学的に認識する抗体。

【請求項１０】　請求項１に記載のポリペプチドの、ＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合性を阻害もしくは増強する化合物のスクリーニング方法であって、請求項１または２に記載のポリペプチド、請求項９に記載の抗体のうち、少なくともいずれか１つを用いることを特徴とするスクリーニング方法。

【請求項１１】　請求項３もしくは４に記載のポリヌクレオチドと相互作用して該ポリヌクレオチドの発現を阻害もしくは増強する化合物のスクリーニング方法であって、請求項３から５のいずれか１項に記載のポリヌクレオチド、請求項６に記載のベクター、請求項７に記載の形質転換体、請求項９に記載の抗体のうち少なくともいずれか１つを用いることを特徴とするスクリーニング方法。

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【請求項１２】　請求項１に記載のポリペプチドのＧＥＦ（グアニンヌクレオチド交換因子：Ｇｕａｎｉｎｅ　ｎｕｃｌｅｏｔｉｄｅ　ＥｘｃｈａｎｇｅＦａｃｔｏｒ）活性を阻害もしくは増強する化合物のスクリーニング方法であって、請求項１または２に記載のポリペプチド、請求項９に記載の抗体のうち少なくともいずれか１つを用いることを特徴とするスクリーニング方法。

【請求項１３】　請求項１０から１２のいずれか１項に記載のスクリーニング方法でスクリーニングされる化合物。

【請求項１４】　請求項１に記載のポリペプチドの、ＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合性を阻害もしくは増強する化合物。

【請求項１５】　請求項３から５のいずれか１項に記載のポリヌクレオチドと相互作用して該ポリヌクレオチドの発現を阻害もしくは増強する化合物。

【請求項１６】　請求項１に記載のポリペプチドのＧＥＦ活性を阻害もしくは増強する化合物。

【請求項１７】　請求項１または２に記載のポリペプチド、請求項３から５のいずれか１項に記載のポリヌクレオチド、請求項６に記載のベクター、請求項７に記載の形質転換体、請求項９に記載の抗体、または請求項１３から１６のいずれか１項に記載の化合物のうち、少なくともいずれか１つを含有することを特徴とする、大腸腫瘍の治療に用いる医薬組成物。

【請求項１８】　請求項１のポリペプチドの発現または活性に関連した疾病の診断手段であって、試料中の（ａ）該ポリペプチドをコードしているポリヌクレオチド、および／または（ｂ）該ポリペプチド、をマーカーとして分析することを含む診断手段。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、大腸癌に関与する癌抑制遺伝子（Ａｄｅｎｏｍａｔｏｕｓ　Ｐｏｌｙｐｏｓｉｓ　Ｃｏｌｉ：ＡＰＣ）がコードするポリペプチドに対する結合能、特にＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有する新規な蛋白質（以下、Ｍ１と呼称する）およびポリペプチドに関するものである。さらに詳しくは、新規蛋白質Ｍ１のアミノ酸配列の全部または一部を有するポリペプチド、該ポリペプチドをコードするポリヌクレオチド、該ポリヌクレオチドを含有する組換えベクター、該組換えベクターで形質転換された形質転換体、該形質転換体を使ったペプチドまたはポリペプチドの製造方法、該ペプチドまたはポリペプチドに対する抗体、これらを利用した化合物のスクリー

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ニング方法、該スクリーニングされた化合物、該ポリペプチド若しくは該ポリヌクレオチドに作用する活性阻害化合物または活性賦活化合物、これらに関係する医薬組成物、およびこれらに関係する疾病診断手段に関係する。

【０００２】

【従来の技術】癌抑制遺伝子ＡＰＣは、家族性腺腫性ポリポーシス（ｆａｍｉｌｉａｌ　ａｄｅｎｏｍａｔｏｕｓ　ｐｏｌｙｐｏｓｉｓ：ＦＡＰ）の原因遺伝子として単離され、散発性の大腸癌の７０～８０％では、該ＡＰＣの異常が報告されている。ＡＰＣ遺伝子産物は、２，８４３個のアミノ酸からなる３００ｋＤａの巨大な蛋白質である（Ｃｅｌｌ，８７：１５９－１７０，１９９６）。ＡＰＣ遺伝子産物は、種々の蛋白質との相互作用が知られており、その１つにβ－カテニンがある。β－カテニンは、カドヘリンの細胞質側ドメインに結合して細胞接着に役割を果たすと同時に、発生過程や腫瘍形成において重要な役割を担うＷｎｔ／Ｗｉｎｇｌｅｓｓシグナル伝達経路の重要な構成要素の１つとしても機能している（Ｃｅｌｌ，８６：３９１－３９９，１９９６）（Ｎａｔｕｒｅ，３８２：６３８－６４２，１９９６）。β－カテニンは、一種の癌遺伝子産物で、ＡＰＣ遺伝子産物は、β－カテニンの機能を抑制することにより癌抑制機能を発揮していると考えられている（Ｓｃｉｅｎｃｅ，２７５：１７８４－１７８７，１９９７）（Ｓｃｉｅｎｃｅ，２７５：１７８７－１７９０，１９９７）（Ｓｃｉｅｎｃｅ，２７５：１７９０－１７９２，１９９７）。その他、ＡＰＣ遺伝子産物は、ＧＳＫ－３ｂ、Ａｘｉｎもしくはコンダクチン／Ａｘｉｌとの相互作用が知られている（Ｓｃｉｅｎｃｅ，２８０：５９６－５９９，１９９８）（Ｃｕｒｒｅｎｔ　Ｂｉｏｌｏｇｙ，８：５７３－５８１，１９９８）（Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．，２７３：１０８２３－１０８２６，１９９８）（Ｇｅｎｅｓ　Ｃｅｌｌｓ，６：３９５－４０３，１９９８）。また、ＡＰＣ遺伝子産物は、ＥＢ１とｈＤＬＧとの、そのＣ末端を介した相互作用も報告されている（Ｓｃｉｅｎｃｅ，２７２：１０２０－１０２３，１９９６）。さらに、ＡＰＣ遺伝子産物には、蛋白質間の相互作用の役割を担うアルマジロリピートドメインが存在することも知られている。

【０００３】

【発明が解決しようとする課題】本発明が解決しようとする課題は、上記のように多様な物質との相互作用を担う大腸癌の癌抑制遺伝子であるＡＰＣ遺伝子に関与する新規物質を見いだすことであり、該新規物質を癌の制御を目的とする手段として使用することである。より具体的には、本発明の課題はＡＰＣ遺伝子産物との結合能、特にＡＰＣ遺伝子産物由来のアルマジロリピートドメインとの結合能、をもつ新規な物質（Ｍ１）を提供することであり、それに伴い有用性ある新規物質（Ｍ１）由来

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のポリペプチドを提供することである。また本発明の別の課題は、Ｍ１由来のポリペプチドをコードするポリヌクレオチドを提供し、遺伝子工学手法による、Ｍ１由来のポリペプチドの製造法を提供することである。さらに本発明の別の課題は、Ｍ１由来のポリペプチドに対する抗体を提供することである。その他の本発明の課題は、上記のものを利用してＭ１の有する作用の阻害剤・拮抗剤・賦活剤のスクリーニングをおこなうことであり、スクリーニングされた化合物を提供することであり、またこれらを利用した大腸腫瘍に用いる医薬組成物・診断手段を提供することである。

【０００４】

【解決するための手段】課題解決のため、本発明者は、ヒト胎児脳ｃＤＮＡライブラリーからＡＰＣ遺伝子産物のアルマジロリピートドメイン（以下、ａｒｍと呼称することもある）に結合する新規蛋白質Ｍ１のｃＤＮＡを２ハイブリッドスクリーニング法を用いて同定し、その塩基配列および該新規蛋白質Ｍ１のｃＤＮＡがコードするアミノ酸配列を決定し、本発明を完成した。

【０００５】すなわち、本発明は下記の群より選ばれるポリペプチド；①配列表の配列番号１に記載のアミノ酸配列で示されるポリペプチド、②前記①のポリペプチドのアミノ酸配列を含有するポリペプチド、③前記①のポリペプチドと少なくとも約７０％のアミノ酸配列上の相同性を有しかつ大腸癌の癌抑制遺伝子（Ａｄｅｎｏｍａｔｏｕｓ　Ｐｏｌｙｐｏｓｉｓ　Ｃｏｌｉ：ＡＰＣ）の遺伝子産物のアルマジロリピート部位をコードするポリペプチドに対する結合能を有するポリペプチド、および④前記①から③のポリペプチドのアミノ酸配列において１ないし数個のアミノ酸の欠失、置換、付加などの変異あるいは誘発変異を有し、かつＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有するポリペプチド；配列表の配列番号１に記載のアミノ酸配列の少なくとも５個のアミノ酸配列を有し、かつＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有するポリペプチド；本発明のポリペプチドをコードするポリヌクレオチドまたはその相補鎖；本発明のポリヌクレオチドまたはその相補鎖とストリンジェントな条件下でハイブリダイゼーションするポリヌクレオチド；本発明のポリヌクレオチドまたはその相補鎖の塩基配列のうち少なくとも１５個の連続した塩基配列で示されるポリヌクレオチドであって、該ポリヌクレオチドの転写によって発現されるポリペプチドがＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合能を有する、ポリヌクレオチド；本発明のポリヌクレオチドを含有する組換えベクター；本発明の組換えベクターで形質転換された形質転換体；本発明の形質転換体を培養する工程を含む、本発明のポリペプチドの製造方法；本発明のポリペプチドのＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合性を阻害もしくは増強する化合物のスクリーニング方法

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であって、本発明のポリペプチド、本発明の抗体のうち少なくともいずれか１つを用いることを特徴とするスクリーニング方法；本発明のポリヌクレオチドと相互作用して該ポリヌクレオチドの発現を阻害もしくは増強する化合物のスクリーニング方法であって、本発明のポリヌクレオチド、本発明のベクター、本発明の形質転換体、本発明の抗体のうち少なくともいずれか１つを用いることを特徴とするスクリーニング方法；本発明のポリペプチドのＧＥＦ（グアニンヌクレオチド交換因子：Ｇｕａｎｉｎｅ ｎｕｃｌｅｏｔｉｄｅ Ｅｘｃｈａｎｇｅ Ｆａｃｔｏｒ）活性を阻害もしくは増強する化合物のスクリーニング方法であって、本発明のポリペプチド、本発明の抗体のうち少なくともいずれか１つを用いることを特徴とするスクリーニング方法；本発明のスクリーニング方法でスクリーニングされる化合物；本発明のポリペプチドのＡＰＣ遺伝子産物のアルマジロリピート部位に対する結合性を阻害もしくは増強する化合物；本発明のポリヌクレオチドと相互作用して該ポリヌクレオチドの発現を阻害もしくは増強する化合物；本発明のポリペプチドのＧＥＦ活性を阻害もしくは増強する化合物；本発明のポリペプチド、本発明のポリヌクレオチド、本発明のベクター、本発明の形質転換体、本発明の抗体、または本発明の化合物のうち少なくともいずれか１つを含有することを特徴とする大腸腫瘍の治療に用いる医薬組成物；本発明のポリペプチドの発現または活性に関連した疾病の診断手段であって、試料中の（ａ）該ポリペプチドをコードしているポリヌクレオチド、および／または（ｂ）該ポリペプチド、をマーカーとして分析することを含む診断手段、を提供する。

【０００６】

【発明の実施の形態】（新規Ｍ１）本発明において提供される新規蛋白質Ｍ１をコードするポリヌクレオチドは、ヒトの胎児脳ｃＤＮＡライブラリーから、ＡＰＣ遺伝子産物のアルマジロリピートドメインを使い、２ハイブリッドスクリーニング法により、新規なアミノ酸配列を有する物質として、そのｃＤＮＡが取得されたものである。本発明の新規蛋白質Ｍ１のｃＤＮＡは、６１９個のアミノ酸からなる蛋白質をコードし、既知のＤｂｌファミリー（低分子量Ｇ蛋白質Ｒｈｏファミリーに作用するＧＤＰ解離促進蛋白質の１つである）（Ｃｕｒｒｅｎｔ Ｏｐｉｎｉｏｎ ｉｎ ＣｅｌｌＢｉｏｌｏｇｙ，８：２１６－２２２，１９９６）に類似のドメイン構造を有していた。遺伝子データベース（Ｔｈｅ Ｎａｔｉｏｎａｌ Ｃｅｎｔｅｒ ｆｏｒ Ｂｉｏｔｅｃｈｎｏｌｏｇｙ Ｉｎｆｏｒｍａｔｉｏｎ）を検索したところ、本発明の新規蛋白質Ｍ１のｃＤＮＡは、Ｄｂｌファミリーの１つである既知物質ＫＩＡＡ０４２４と約７３％の相同性を有することが判明した。ＫＩＡＡ０４２４は、本発明の新規蛋白質Ｍ１とは、該Ｍ１のＮ末端領域を欠如する点に最も大きな差異を有する。両者は、Ｄｂ

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ｌ相同（ＤＨ）ドメイン、プレックストリン（Ｐｒｅｃｋｓｔｒｉｎ）相同（ＰＨ）ドメイン、Ｓｒｃ相同３（ＳＨ３）ドメインを担持する点において同一である。また、マウスを用いた新規蛋白質Ｍ１の組織分布研究において、該Ｍ１のｍＲＮＡが脳に高レベルで発現しており、他の臓器でも低レベルで発現していることを確認した。

【０００７】新規蛋白質Ｍ１のペプチド断片（配列表、配列番号１のアミノ酸７３～１２６）を使用して作製した抗体は、グルタチオンーＳートランスフェラーゼ（ＧＳＴ）との融合蛋白として得た新規蛋白質Ｍ１に対し強い反応性を示した。また、該作製した抗体を用い、脳に存在する新規物質Ｍ１が、約８５ｋＤａの蛋白質であることを抗原抗体反応を利用した測定系で確認した。

【０００８】また、新規蛋白質Ｍ１とＡＰＣ遺伝子産物との直接的な相互作用を確認するための実験において、グルタチオンーＳートランスフェラーゼ（ＧＳＴ）との融合蛋白として得たＡＰＣ遺伝子産物のアルマジロリピートドメイン（ＡＰＣ－ａｒｍ）は、ＧＳＴ融合Ｍ１断片（ＧＳＴ－Ｍ１－Ｍ）と相互作用したが、ＧＳＴ単独とは反応せず、同様に、Ｍ１－Ｍは、ＧＳＴ－ＡＰＣ－ａｒｍとは反応したが、ＧＳＴ融合β－カテニンのアルマジロリピートドメイン又はＧＳＴ単独とは反応しないことを確認した。すなわち、新規蛋白質Ｍ１はＡＰＣ遺伝子産物と、該ＡＰＣ遺伝子産物のアルマジロリピートドメインを介して結合していると推定される。また、ラット胎児脳の溶解物（ｌｙｓａｔｅ）を抗ＡＰＣ抗体で免疫沈降し、ついで抗Ｍ１抗体でイムノブロットすることにより、ＡＰＣ遺伝子産物と本発明のＭ１が共沈殿することが判明した。この反応において、抗Ｍ１抗体を、抗原性を保持するＭ１断片で前処理すると、Ｍ１とＡＰＣ遺伝子産物の共沈殿が阻害された。すなわち、Ｍ１とＡＰＣ遺伝子産物とは生体内で結合していると考えられる。また、Ｍ１とＡＰＣ遺伝子産物の結合部位を２ハイブリッド法を用いて確認したところ、少なくともＭ１のアミノ酸７３～１２６で示される領域に結合部位が存在することが推定された。このことは、Ｍ１のＳＨ３ドメインの上流域に、ＡＰＣ遺伝子産物のアルマジロリピートドメインとの結合部位が存在することを意味する。ＫＩＡＡ０４２４は、このような領域をもたないので、ＡＰＣ遺伝子産物との反応性がない。

【０００９】さらに、新規蛋白質Ｍ１はその作用として、Ｒｈｏファミリーの１つであるＲａｃに特異的なＧＥＦ活性をもつことを確認した。つまり、新規蛋白質Ｍ１は、Ｒａｃに結合しＧＤＰ／ＧＴＰ交換反応を促進してＲａｃを活性化し、Ｒａｃの関与する細胞情報伝達の下流に位置するＮＦκＢ、ｃ－ｊｕｎ、ＳＲＥ等に作用する。また、Ｒａｃの生理機能である、細胞のラメリポディア（葉状仮足）や細胞膜のラッフリングを誘導する可能性もあり、細胞接着への関与が推定される。

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【００１０】ＡＰＣ遺伝子産物の細胞内局在については、細胞が大腸絨突起先端へクリプトから移動する際に、移動する細胞の微小管先端部位に集積していることが報告されている（Ｊ．Ｃｅｌｌ　Ｂｉｏｌ．，１３４：１６５－１７９，１９９６）が、本発明の新規蛋白Ｍ１も同様の部位に集積していることを見出した。このことから、本発明の新規蛋白質Ｍ１が、大腸絨突起における細胞移動制御の鍵を握っている可能性がある。

【００１１】（ポリペプチド）本発明の新規蛋白質Ｍ１は、配列表の配列番号１に示すアミノ酸配列からなるポリペプチドである。さらに本発明のポリペプチドは、この配列表の配列番号１に示すポリペプチドの部分配列を有するポリペプチドから選択される。その選択されるポリペプチドは、配列表の配列番号１に示すポリペプチドと、好ましくは約７０％以上、より好ましくは約８０％以上、さらに好ましくは約９０％をこえる相同性を有する。この相同性をもつポリペプチドの選択は、例えばＡＰＣ遺伝子産物のアルマジロリピートドメインとの結合性を指標にして行うことができる。

【００１２】アミノ酸配列の相同性を決定する技術は、自体公知であり、例えばアミノ酸配列を直接決定する方法、推定されるポリヌクレオチドの塩基配列を決定後これにコードされるアミノ酸配列を推定する方法等を使用することができる。

【００１３】本発明のポリペプチドは、配列表の配列番号１に示すアミノ酸配列からなるポリペプチドの、部分配列を有するポリペプチドから選択されるアミノ酸配列を試薬・標準物質・免疫原として利用できる。その最小単位としては、少なくとも約５個以上、好ましくは少なくとも約８～１０個以上、さらに好ましくは少なくとも約１１～１５個以上のアミノ酸で構成されるアミノ酸配列からなり、免疫学的にスクリーニングしうるポリペプチドを本発明の対象とする。

【００１４】さらに、このように特定されたポリペプチドをもとにして、ＡＰＣ遺伝子産物のアルマジロリピートドメインとの結合性を指標とすることにより、１ないし数個のアミノ酸の欠失・置換・付加などの変異あるいは誘発変異を有するアミノ酸配列からなるポリペプチドも提供することができる。欠失・置換・付加あるいは挿入の手段は自体公知であり、例えばＵｌｍｅｒの技術（Ｓｃｉｅｎｃｅ，２１９：６６６，１９８３）を利用することが出来る。さらに、これら利用できるペプチドは、その構成アミノ基もしくはカルボキシル基などを修飾するなど、機能の著しい変更を伴わない程度に改変が可能である。

【００１５】本発明のポリペプチドは、それら自体で、新規蛋白質Ｍ１の機能を調節するための医薬組成物に使用できる。また、本発明のポリペプチドは、新規蛋白質Ｍ１の機能を調節しうる化合物、例えば、阻害剤、拮抗剤、賦活剤等を得るためのスクリーニングや、新規蛋白質Ｍ１に対する抗体の取得に用いることができる。さらに、本発明のポリペプチドは、試薬・標準品としても使用可能である。

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【００１６】（ポリヌクレオチド）本発明のポリヌクレオチドおよびその相補鎖は、配列表の配列番号１に記載のアミノ酸配列をコードする、配列表の配列番号２のポリヌクレオチドおよび該ポリヌクレオチドに対する相補鎖、これらのポリヌクレオチドとストリンジェントな条件下でハイブリダイゼーションするポリヌクレオチド、およびこれらのポリヌクレオチドのうち少なくとも１５個の連続した塩基配列を有しかつコードするペプチドがＡＰＣ遺伝子産物のａｒｍドメインとの結合能を有するポリヌクレオチド、を意味する。ポリヌクレオチドとしてＤＮＡを代表例にとると、「ＤＮＡにストリンジェントな条件下でハイブリダイズするＤＮＡ」は、例えば前述のＭｏｌｅｃｕｌｅｒ　Ｃｌｏｎｉｎｇに記載の方法によって得ることができる。ここで、「ストリンジェントな条件下でハイブリタイズする」とは、例えば、６×ＳＳＣ、０．５％ＳＤＳおよび５０％ホルムアミドの溶液中で４２℃にて加温した後、０．１×ＳＳＣ、０．５％ＳＤＳの溶液中で ６８℃にて洗浄する条件でも依然として陽性のハイブリタイズのシグナルが観察されることを表す。

【００１７】本発明のポリヌクレオチドは、配列表の配列番号１に記載のアミノ酸配列をコードする、配列表の配列番号２のポリヌクレオチドの情報から選択される相同鎖および相補鎖を意味し、指定されたヌクレオチド配列の領域に対応する少なくとも約１５～２０個以上の配列からなるポリヌクレオチド配列及び該相補鎖を意味する。この有用なポリヌクレオチド配列の決定は、公知の蛋白質発現系、例えば無細胞蛋白質発現系を利用して簡易に発現蛋白質の確認を行い、その生理活性特にＡＰＣ遺伝子産物のアルマジロリピートドメインとの結合性を指標にして選別することにより行うことができる。無細胞蛋白質発現系としては、例えば胚芽、家兎網状赤血球等由来のリボソーム系の技術を利用できる（Ｎａｔｕｒｅ、１７９、１６０～１６１、１９５７）。

【００１８】これらのポリヌクレオチドは、いずれも本発明の新規蛋白質Ｍ１および本発明のポリペプチドの製造に有用な遺伝子情報を提供するものであり、これらをコードする遺伝子等の核酸、またはｍＲＮＡ検出のためのプローブもしくはプライマーとして、あるいは遺伝子発現を調節するためのアンチセンスオリゴマーとして使用することができる。例えば、本発明のポリヌクレオチドをアンチセンスとして使用する場合、他の既知蛋白質、例えばＤｂｌファミリーの１つであるＫＩＡＡ０４２４等、とのコンセンサス配列領域以外の新規蛋白質Ｍ１に固有な領域のヌクレオチド配列を用いることにより、Ｍ１の発現が特異的に阻害される。さらに、本発明のポリヌクレオチドは、核酸に関する試薬・標準品とし

ても利用できる。

【００１９】（形質転換体）上記のような無細胞蛋白質発現系以外にも、大腸菌、酵母、枯草菌、昆虫細胞、動物細胞等の自体公知の宿主を利用した遺伝子組換え技術によって、本発明からなる新規蛋白質Ｍ１およびその由来物からなるポリペプチドを提供可能である。本発明の具体例においては、ＣＯＳ－７細胞を利用したが、無論これに限定されるものではない。

【００２０】形質転換は、自体公知の手段を応用することができ、例えばレプリコンとして、プラスミド、染色体、ウイルス等を利用して宿主の形質転換を行う。より好ましい系としては、遺伝子の安定性を考慮するならば、染色体内へのインテグレート法があげられるが、簡便には核外遺伝子を用いた自律複製系を利用する。ベクターは、宿主の種類により選択され、発現目的の遺伝子配列と複製そして制御に関する情報を担持した遺伝子配列とを構成要素とする。構成要素は宿主が原核細胞か真核細胞かによって選択し、プロモーター、リボソーム結合部位、ターミネーター、シグナル配列、エンハンサー等を自体公知の方法によって組合せて使用する。

【００２１】形質転換体は、自体公知の各々の宿主の培養条件に最適な条件を選択して培養することにより、本発明のポリペプチドの製造に用いることができる。培養は、発現産生される新規蛋白質Ｍ１およびその由来物からなるポリペプチドの生理活性、特にＡＰＣ遺伝子産物のアルマジロリピートドメインとの結合性を指標にして行ってもよいが、培地中の形質転換体量を指標にして継代培養またはバッチによって行う。

【００２２】（新規蛋白質Ｍ１およびその由来物の回収）培地からの新規蛋白質Ｍ１およびその由来物からなるポリペプチドの回収は、ＡＰＣ遺伝子産物のアルマジロリピートドメインとの結合性を指標にして、分子篩、イオンカラムクロマトグラフィー、アフィニティクロマトグラフィー等を組合せるか、溶解度差にもとづく硫安、アルコール等の分画手段によっても精製回収できる。より好ましくは、アミノ酸配列の情報に基づき、該アミノ酸配列に対する抗体を作成し、ポリクローナル抗体またはモノクローナル抗体によって、特異的に吸着回収する方法を用いる。

【００２３】（抗体）抗体は、本発明の新規蛋白質Ｍ１およびその由来物からなるポリペプチドの抗原決定基を選別し、作成する。抗原決定基は、少なくとも５個、より好ましくは少なくとも８～１０個のアミノ酸で構成される。このアミノ酸配列は、必ずしも配列表の配列番号１と相同である必要はなく、蛋白質の立体構造上の外部への露出部位であればよく、露出部位が不連続部位であれば、該露出部位について連続的なアミノ酸配列であることも有効である。実施例では、アミノ酸配列の７３位～１２６位の断片を免疫原として利用した。抗体は、免疫学的に新規蛋白質Ｍ１およびその由来物からなるポリペプチドを認識する限り特に限定されない。この認識の有無は、公知の抗原抗体結合反応によって決定する。

【００２４】抗体を産生するためには、本発明の新規蛋白質Ｍ１およびその由来物からなるポリペプチドを、アジュバントの存在または非存在下で、単独または担体に結合して、動物に対して体液性応答および／または細胞性応答等の免疫誘導を行う。担体は、自身が宿主に対して有害作用をおこさなければ特に限定されず、例えばセルロース、重合アミノ酸、アルブミン等が例示される。免疫する動物としては、マウス、ラット、兎、やぎ、馬等が好適に用いられる。ポリクローナル抗体は、自体公知の血清からの抗体回収法によって取得する。好ましい手段としては、免疫アフィニティークロマトグラフィー法である。実施例においては、ＧＳＴ－Ｍ１を結合させたアフィニティークロマトグラフィーにより、抗Ｍ１抗体を精製した。

【００２５】モノクローナル抗体を生産するためには、上記の免疫手段が施された動物から抗体産生細胞を回収し、自体公知の永久増殖性細胞への形質転換手段を導入することによって行われる。

【００２６】ポリクローナル抗体またはモノクローナル抗体は、直接本発明からなる新規蛋白質Ｍ１と結合し、その活性を制御可能であり、ＡＰＣ遺伝子産物と新規蛋白質Ｍ１との相互作用系の制御を容易に行うことができる。そのため、ＡＰＣ遺伝子産物と新規蛋白質Ｍ１が関連する疾患の治療・予防のために有用である。

【００２７】（スクリーニング）かくして調製された新規蛋白質Ｍ１およびその由来物からなるポリペプチド、これらをコードするポリヌクレオチドおよびその相補鎖、これらのアミノ酸配列および塩基配列の情報に基づき形質転換させた細胞、並びに新規蛋白質Ｍ１およびその由来物からなるポリペプチドを免疫学的に認識する抗体は、単独または複数手段を組合せることによって、新規蛋白質Ｍ１およびその由来物からなるポリペプチドとＡＰＣ遺伝子産物との結合性、新規蛋白質Ｍ１のＧＥＦ活性等の機能、または新規蛋白質Ｍ１の発現に対する阻害剤もしくは賦活剤のスクリーニングに有効な手段を提供する。すなわち、本発明のポリペプチド、本発明の抗体の少なくともいずれか１つを用いることで、本発明のポリペプチドとＡＰＣ遺伝子産物との結合性を阻害もしくは増強する化合物を得るためのスクリーニング方法が、本発明のポリヌクレオチド、本発明のベクター、本発明の形質転換体、本発明の抗体の少なくともいずれか１つを用いることで本発明のポリヌクレオチドと相互作用し該ポリヌクレオチドの発現を阻害もしくは増強する化合物のスクリーニング方法が、本発明のポリペプチド、本発明の抗体の少なくともいずれか１つを用いることで本発明のポリペプチドのＧＥＦ活性等の機能を阻害もしくは増強する化合物のスクリーニング方法が提供可能である。例えば、ポリペプチドの立体構造に基づくド

ラッグデザインによる拮抗剤の選別、蛋白質発現系を利用した遺伝子レベルでの発現調整剤の選別、抗体を利用した抗体認識物質の選別等が、自体公知の医薬品スクリーニングシステムにおいて利用可能である。

【００２８】（化合物、医薬組成物）上記のスクリーニング方法で得られた化合物は、新規蛋白質Ｍ１およびその由来物からなるポリペプチドとＡＰＣ遺伝子産物との相互作用、または新規蛋白質Ｍ１のＧＥＦ活性等の機能を調節する阻害剤、拮抗剤、賦活剤等の候補化合物として利用可能である。また、遺伝子レベルでの新規蛋白質Ｍ１およびその由来物からなるポリペプチドの発現に対する阻害剤、拮抗剤、賦活剤等の候補化合物としても利用可能である。上記の阻害剤、拮抗剤、賦活剤等の候補化合物としては、蛋白質、ポリペプチド、抗原性を有さないポリペプチド、低分子化合物等が挙げられ、好ましくは低分子化合物である。

【００２９】かくして選別された候補化合物は、生物学的有用性と毒性のバランスを考慮して選別することによって、大腸腫瘍の治療に用いる医薬組成物として調製可能である。また、本発明からなる新規蛋白質Ｍ１およびその由来物からなるポリペプチド、これらをコードするポリヌクレオチドおよびその相補鎖、これらの塩基配列を含むベクター並びに、新規蛋白質Ｍ１およびその由来物からなるポリペプチドを免疫学的に認識する抗体は、それら自体が、新規蛋白質Ｍ１とＡＰＣ遺伝子産物との相互作用に対する阻害・拮抗・賦活等の機能を有する、大腸腫瘍の治療に用いる医薬手段として使用できる。ここで、大腸腫瘍とは、良性腫瘍ならびに悪性腫瘍を含み、具体的には、家族性腺腫性ポリポーシス（ＦＡＰ）および大腸癌が挙げられる。なお、製剤化にあたっては、自体公知のポリペプチド、蛋白質、ポリヌクレオチド、抗体等、各対象に応じた製剤化手段を導入すればよい。

【００３０】本発明からなる新規蛋白質Ｍ１およびその由来物からなるポリペプチド、これらをコードするポリヌクレオチドおよびその相補鎖、これらの塩基配列を含むベクター並びに、新規蛋白質Ｍ１およびその由来物からなるポリペプチドを免疫学的に認識する抗体は、本発明のポリペプチドの発現またはその活性が関連する疾患、例えば、本発明の新規蛋白質Ｍ１の発現またはＡＰＣ遺伝子産物との相互作用に関連した疾患等の診断手段として使用することができる。特に、大腸腫瘍の診断マーカーおよび／または試薬等の診断手段として有用である。診断は、新規蛋白質Ｍ１をコードしている核酸配列との相互作用・反応性を利用して、相応する核酸配列の存在量を決定すること、および／または新規蛋白質Ｍ１について生体内分布を決定すること、および／または新規蛋白質Ｍ１の試料中での存在量を決定することによって行う。詳しくは、新規蛋白質Ｍ１を診断マーカーとして検定する。その測定法は、自体公知の抗原抗体反応

系、酵素反応系、ＰＣＲ反応系等を利用すればよい。なお、ここで言う手段とは、目的達成のために使用する方法および／または媒体を意味する。すなわち、例えば、診断手段には、診断するための方法、診断に用いる試薬キットなどが含まれる。

【００３１】

【実施例】以下、本発明を実施例に基づき具体的に説明するが、本発明は下記の実施例に限定されない。

（ｃＤＮＡのクローニング）ＡＰＣ遺伝子産物のアルマジロリピートドメイン（以下、ＡＰＣ－ａｒｍと略称することもある）に対する結合能を有する蛋白質を得ることを目的として、2ハイブリッドスクリーニング（ｔｗｏ ｈｙｂｒｉｄ ｓｃｒｅｅｎｓ）（ＣＬＯＮＴＥＣＨ ＭＡＴＣＨＭＡＫＥＲ™ Ｔｗｏ－Ｈｙｂｒｉｄ Ｓｙｓｔｅｍ）を行った。すなわち、ベイト（ｂａｉｔ）として、ヒトＡＰＣ遺伝子産物アルマジロリピートドメイン（アミノ酸残基４４６－８８０）を融合させたＧＡＬ４ ＤＮＡ結合ドメインを含有するプラスミドＧＢＴ９－ＡＰＣを用い、ヒト胎児脳（ｈｕｍａｎ ｆｅｔａｌ ｂｒａｉｎ）ｃＤＮＡライブラリー（Ｃｌｏｎｔｅｃｈ）を対象としてスクリーニングした。該ｃＤＮＡライブラリーとベイトとを、レポーター遺伝子としてｈｉｓ３とｌａｃＺとを導入した酵母にトランスフェクションし、β－ｇａｌアッセイおよびＨＩＳ栄養要求性を指標として、陽性クローンを検出した。

【００３２】形質転換体１．１×１０^{7}個から、１つの陽性クローンを得た。この得られたクローンから、該クローンで発現されているＡＰＣ－ａｒｍに結合するポリペプチドをコードするｃＤＮＡ断片の塩基配列をＤＮＡシークエンスにより決定したところ、新規な配列であった。得られたｃＤＮＡ断片の下流領域の配列は’Ｍａｒａｔｈｏｎ’－ｒｅａｄｙ ヒト脳ｃＤＮＡ（Ｃｌｏｎｔｅｃｈ）を用いて3’ＲＡＣＥシステム（Ｃｌｏｎｔｅｃｈ）により取得した。プライマーは5’－ＣＧＡＣＡＴＣＴＧＣＧＡＧＧＧＣＴＡＣＧＴＣＣＧＧ－3’を用いた。また上記で得られたｃＤＮＡ断片の配列の一部（配列表、配列番号２の塩基番号９７－２６９）をプローブとして、ヒトゲノムライブラリー（Ｃｌｏｎｔｅｃｈ）について、ジゴキシゲニン（ｄｉｇｏｘｉｇｅｎｉｎ；ＤＩＧ）標識プローブを用いたハイブリダイゼーションによりスクリーニングした。その結果、配列表、配列番号２の塩基番号１－８１を含む２つのオーバーラップするクローンを得、目的とするＡＰＣ－ａｒｍに結合する蛋白質の完全長ｃＤＮＡの配列を決定した。

【００３３】（アミノ酸配列）上記方法で得られた、配列表の配列番号２に示すｃＤＮＡは新規な塩基配列を有していた。該ｃＤＮＡをもとに、その塩基配列の翻訳によって、新規蛋白質Ｍ１の推定アミノ酸配列、すなわち配列表の配列番号１に示すアミノ酸残基６１９の配列が得られた（図１Ａ）。

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【００３４】（既存蛋白質との相同性）該新規蛋白質Ｍ１の推定アミノ酸配列を用いて、既存のｄａｔａｂａｓｅ（Ｇｅｎｂａｎｋ）に対してＢＬＡＳＴ（Ｔｈｅ Ｎａｔｉｏｎａｌ Ｃｅｎｔｅｒｆｏｒ Ｂｉｏｔｅｃｈｎｏｌｏｇｙ Ｉｎｆｏｒｍａｔｉｏｎ）を用いた相同性検索を行ったところＤｂｌファミリーのサブファミリーメンバーの１つであるＫＩＡＡ０４２４と７３％の相同性を認めた（図２Ｂ、Ｃ）。両者は、Ｄｂｌ相同（Ｄｂｌ ｈｏｍｏｌｏｇｙ；ＤＨ）ドメイン、プレックストリン相同（Ｐｒｅｃｋｓｔｒｉｎ ｈｏｍｏｌｏｇｙ；ＰＨ）ドメイン、Ｓｒｃ相同３（ＳＨ３）ドメインを保持する点において同一であるが、ＫＩＡＡ０４２４には、新規蛋白質Ｍ１のＮ末端領域は存在しないことが判明した。この結果、配列表の配列番号１に示すアミノ酸配列を有する本発明の蛋白質Ｍ１は新規蛋白質であることが確認された。

【００３５】（発現組織の確認）次に配列表の配列番号１の推定アミノ酸配列で示される新規蛋白質Ｍ１の、ヒト組織における発現を、ノザンブロット解析により確認した。多種のヒト組織から得たｐｏｌｙ（Ａ）＋ＲＮＡをブロットしたフィルターをＣｌｏｎｔｅｃｈ社より入手し、ＤＩＧ標識したＭ１のｃＤＮＡプローブ、５’－ＧＡＣＣＡＣＡＣＴＧＣＣＡＴＣＧＣＴＧ－３’および５’－ＴＧＴＡＧＴＴＴＡＣＣＡＡＧＧＡＣＣＧ－３’でハイブリダイゼーションした。その結果、脳に高レベルで発現していることを確認した。また、ｔｉｓｓｕｅ ｂｌｏｔｓによる解析では、脳以外に、睾丸でも存在を確認した。さらに、腎由来の細胞でも発現を確認した。

【００３６】（抗体の産生）Ｍ１に対する抗体は、ＮＺＷウサギをＭ１のアミノ酸７３－１２６を含むペプチドを用いて公知の方法で免疫し調製した。ＡＰＣに対する抗体は、ＮＺＷウサギをＡＰＣ遺伝子産物（以下ＡＰＣと略称することもある）のアミノ酸１１２２－１７２９を含むペプチドを用いて公知の方法で免疫して調製した。ＡＰＣのＮ末端領域に対するマウスモノクローナル抗体は公知の方法で調製した（Ｍｉｙａｓｈｉｒｏ ｅｔ ａｌ．１９９５）。抗体は、それぞれ免疫に用いた抗原を結合させたアフィニティーカラムを使用してアフィニティークロマトグラフィーを行うことにより精製した。精製したウサギポリクローナル抗Ｍ１抗体とＧＳＴ融合Ｍ１（ＧＳＴ－Ｍ１）との結合反応性を調べたところ、得られた抗体は、ＧＳＴ－Ｍ１に強い反応性を示した。

【００３７】（Ｍ１の発現）本発明の新規蛋白質Ｍ１を発現させるために、発現プラスミドｐｃＤＮＡ３．１（＋）のＥｃｏＲＩ／ＮｏｔＩ部位にＭ１のｃＤＮＡを組み込み、ＣＯＳ－７細胞にトランスフェクションした。新規蛋白質Ｍ１の発現確認のため、Ｍ１ｃＤＮＡを組み込んだベクター、コントロールベクター、ＨＡ－タグで標識したＭ１ｃＤＮＡを組み込んだベクター、ＨＡ－タグを組み込んだベクターを、ＣＯＳ－７細胞にトランスフェクションした。培養した各形質転換体の溶解物、およびＭ１の存在が確認されているラット胎児脳の溶解物について、上記の様に取得した抗Ｍ１抗体、および抗ＨＡ抗体を用いて免疫沈降し、ＳＤＳ－ＰＡＧＥにより分画し、次いで抗Ｍ１抗体および抗ＨＡ抗体を用いてイムノブロットを行った。Ｍ１ｃＤＮＡを組み込んだベクター（レーン３）、ＨＡ－タグで標識したＭ１ｃＤＮＡを組み込んだベクター（レーン６、８）で形質転換したＣＯＳ－７細胞およびラット胎児脳（レーン１）では明らかに新規蛋白質の発現が認められ、その分子量は約８５ｋＤａであることが判明した（図２）。また、用いた抗Ｍ１抗体を抗原で事前処理しておく（図２中、ｐｅｐ．±で表示）と、新規蛋白質は検出されなかった。すなわち、抗Ｍ１抗体で認識される新規蛋白質Ｍ１が発現されたことが確認された。

【００３８】（Ｍ１とＡＰＣとのｉｎ ｖｉｖｏにおける結合の解析）ラット胎児脳溶解物について、上記で作製した新規蛋白質Ｍ１、およびＡＰＣに対するウサギポリクローナル抗体、ならびにβ－カテニンに対するマウスモノクローナル抗体（Ｔｒａｎｓｄｕｃｔｉｏｎ Ｌａｂｏｒａｔｏｒｉｅｓ）を用いて免疫沈降し、ＳＤＳ－ＰＡＧＥにより各沈降物を分離後、各抗体を用いてイムノブロットを行った（図３）。レーン１および２はラット胎児脳の溶解物を抗Ｍ１抗体で、レーン３および４は抗ＡＰＣ抗体で、レーン５および６は抗β－カテニン抗体で免疫沈降した結果を、レーン１、３、５は、予め各抗体を対応する抗原で吸収して用いた結果（図３中、ｐｅｐ．±で表示）を示す。抗Ｍ１抗体で免疫沈降したＭ１が抗ＡＰＣ抗体もしくは抗β－カテニン抗体で検出され、抗ＡＰＣ抗体で免疫沈降したＡＰＣ遺伝子産物が抗Ｍ１抗体で検出され、抗β－カテニン抗体で免疫沈降したβ－カテニンが抗Ｍ１抗体で検出されることから、新規蛋白質Ｍ１はｉｎ ｖｉｖｏにおいてもＡＰＣおよびβ－カテニンと結合していることが明らかとなった。

【００３９】（Ｍ１とＡＰＣの相互作用部位の解析）新規蛋白質Ｍ１の、ＡＰＣ結合ドメインの解析を行うため、Ｍ１の様々な欠失変異体（ｄｅｌｅｔｉｏｎ ｍｕｔａｎｔ）を公知の方法で作製し、ＡＰＣとの結合領域を酵母（ｙｅａｓｔ）を用いた２ハイブリッドシステムにより調べた。具体的には、Ｍ１の欠失変異体を、下記の特異的なプライマーを用いＰＣＲを行って、ｐＧＡＤ４２４にクローニングし、ＧＡＬ４活性化ドメインとの融合体を作成した。

５’－ＡＴＴＴＡＴＴＧＴＡＧＴＴＴＡＣＣＡＡＧＧＡＣ－３’
５’－ＴＧＣＧＣＴＧＡＡＧＣＡＣＴＣＴＧＧＧＡＣ－３’

15　　16

5' −GACCACACTGCCATCGCTGATG−3'
5' −CCTCAGCCGAACGAAGCTGGCTG−3'
5' −CTTGCTGCTCTGCGCCTCCGC−3'
5' −GTGAATCAGGACGAGCCCGCG−3'
5' −GATGTTCCCGAAGATGGTACG−3'
5' −ATGCCTGATGGAGCTCTGGAC−3'

ついで、該ＧＡＬ４活性化ドメインとの融合体を２ハイブリッドシステムにおいてＨＩＳ３ ａｕｘｏｔｒｏｐｈｙとβ−ｇａｌ リポーター活性を用いて、その相互作用を試験した。その結果、図４に示すように、ＳＨ３の上流の領域にＡＰＣ結合部位が存在することが判明した。

【００４０】（Ｍ１とＲｈｏファミリー低分子量Ｇ蛋白質との結合解析）新規蛋白質Ｍ１は、Ｄｂｌファミリーのサブファミリーの１つであるＫＩＡＡ０４２４と高い相同性を有する。Ｄｂｌファミリーは低分子量Ｇ蛋白質の１つであるＲｈｏファミリーに作用するＧＤＰ解離促進蛋白質である。新規蛋白質Ｍ１と、ＫＩＡＡ０４２４との相同性に着目し、新規蛋白質Ｍ１の機能解析のため、Ｒｈｏファミリー低分子量Ｇ蛋白質（ｓｍａｌｌ Ｇ ｐｒｏｔｅｉｎ；ＲｈｏＡ、Ｒａｃ１、ＣＤＣ４２）との結合を調べた。ニッケルビーズに吸着させた新規蛋白質Ｍ１をＲｈｏＡ、Ｒａｃ１およびＧＳＴ−ＣＤＣ４２と、０．１%ＮＰ−４０を含むＥ１Ａ緩衝液〔５０ｍＭ ＨＥＰＥＳ，ｐＨ７．０，１５０ｍＭＮａＣｌ，５０ｍＭ ＮａＦ，５ｍＭ ＥＤＴＡ，１ｍＭＤＴＴ，５０μｇ／ｍＬ ｐｈｅｎｙｌｍｅｔｈｙｌｓｕｌｐｈｏｎｙｌ ｆｌｕｏｒｉｄｅ（ＰＭＳＦ），１μｇ／ｍｌ ロイペプチン，１μｇ／ｍｌアプロチニン〕中で４℃にて１時間混合し、共沈殿物をイムノブロッティングで検出した。図５に示すように、陽性コントロールとして使用したＤｂｌは用いた全てのＲｈｏファミリー低分子量Ｇ蛋白質と結合するが、新規蛋白質Ｍ１はＲｈｏＡおよびＲａｃ１とは結合したが、ＣＤＣ４２とは結合しなかった。

【００４１】（Ｍ１のＧＥＦ活性）次に、新規蛋白質Ｍ１のＧＥＦ活性について検討した。低分子量Ｇ蛋白質からのＧＤＰ解離を調べるために使用する［^{3}Ｈ］ＧＤＰ結合型の低分子量Ｇ蛋白質を、２ｐｍｏｌの各低分子量Ｇ蛋白質を０．２μＭ［^{3}Ｈ］ＧＤＰと３０℃で２０分間、導入用緩衝液（ｌｏａｄｉｎｇ ｂｕｆｆｅｒ；２０ｍＭ Ｔｒｉｓ−ＨＣｌ、ｐＨ８．０、３ｍＭ ＭｇＣｌ$_{2}$、１０ｍＭ ＥＤＴＡおよび１ｍＭジチオスレイトール）中でインキュベートすることにより得た。低分子量Ｇ蛋白質からの［^{3}Ｈ］ＧＤＰの解離を防ぐため、３７５ｍＭ ＭｇＣｌ$_{2}$を終濃度が１８ｍＭとなるように加え、直ちに、該混合液を氷冷した。［^{3}Ｈ］ＧＤＰの解離は、反応溶液（２０ｍＭ Ｔｒｉｓ−ＨＣｌ、ｐＨ８．０、６ｍＭ ＭｇＣｌ$_{2}$、３．５ｍＭ ＥＤＴＡおよび１ｍＭ ジチオスレイトール）に２５０倍過剰の非標識ＧＤＰ、ＧＴＰおよびＭ１を加えることにより２５℃で行った（図６Ａ、Ｂ）。次に、低分子量Ｇ蛋白質へのＧＴＰの結合を調べるために使用するＧＤＰ結合型の低分子量Ｇ蛋白質を、２ｐｍｏｌの各低分子量Ｇ蛋白質を０．２μＭ非標識ＧＤＰと３０℃で２０分間、導入用緩衝液（ｌｏａｄｉｎｇ ｂｕｆｆｅｒ）中でインキュベートし、３７５ｍＭ ＭｇＣｌ$_{2}$を終濃度が１８ｍＭとなるように加えることにより得た。［^{35}Ｓ］ＧＴＰγＳの、ＧＤＰ結合型低分子量Ｇ蛋白質への結合は、１０μＭの［^{35}Ｓ］ＧＴＰγＳおよびＭ１を反応溶液に加えることにより、２５℃で実施した（図７Ａ、Ｂ）。解離試験、結合試験の両方において、反応は１ｍｌの氷冷した停止用緩衝液（２０ｍＭ Ｔｒｉｓ−ＨＣｌ、ｐＨ８．０、２５ｍＭ ＭｇＣｌ$_{2}$および１００ｍＭ ＮａＣｌ）を加えて、停止した。希釈した混合溶液をニトロセルロースろ紙で濾過し、フィルターを数回、停止用緩衝液で洗浄した。ろ紙上に捕捉された放射活性をカウントした。タンパク質濃度はウシ血清アルブミン（ＢＳＡ）を標準蛋白質として用いて測定した。

【００４２】図６に示すように、新規蛋白質Ｍ１は、結合しうる低分子量Ｇ蛋白質であるＲａｃ１に作用して、Ｒａｃ１からのＧＤＰ解離を促進したが（図６Ａ）、ＲｈｏＡには結合しないのでＲｈｏＡからのＧＤＰ解離には作用しなかった（図６Ｂ）。また、図７に示すように、新規蛋白質Ｍ１は、Ｒａｃ１へのＧＴＰ結合を促進した（図７Ａ）が、ＲｈｏＡへのＧＴＰ結合には影響しなかった（図７Ｂ）。すなわち、本発明の新規蛋白質Ｍ１は、低分子量Ｇ蛋白質に作用し、ＧＥＦ活性を有する。

【００４３】（Ｍ１のＧＥＦ活性に対するＡＰＣの作用）さらに、様々な濃度のＭ１について、ＡＰＣ−ａｒｍの存在下または非存在下で、２０ｎＭの［^{3}Ｈ］ＧＤＰを結合させた結合型のＲａｃ１からの［^{3}Ｈ］ＧＤＰ解離促進能を３０℃で１５分間インキュベーションして測定した。ＡＰＣ−ａｒｍはＭ１より過剰量、モル比で５倍となるように加えた。図８に示すように、Ｍ１のＧＤＰ解離促進能は、ＡＰＣ−ａｒｍの添加により、増強された。すなわち、Ｍ１は、ＡＰＣと結合することにより、ＧＤＰ解離促進能が増強されることが示唆された。

【００４４】（Ｍ１の細胞内局在）次に、本発明の新規蛋白質Ｍ１の細胞内局在について検討した。まず、Ｍａｄｉｎ−Ｄａｒｂｙ ｃａｎｉｎｅ ｋｉｄｎｅｙ（ＭＤＣＫ）上皮細胞株を１０%ウシ胎児血清（ＦＢＳ）を含むＤｕｌｂｅｃｃｏ’ｓ ｍｏｄｉｆｉｅｄ Ｅａｇ

ｌｅ’ｓ　ｍｅｄｉｕｍ（DMEM）中で培養した。全長のＭ１およびＭ１ΔＮＢ（アミノ酸１２７－６１９）ｃＤＮＡはＣＭＶプロモーターを有する哺乳動物発現ベクターｐｃＤＮＡ３．１（＋）中にサブクローン化した。プラスミドＤＮＡ、ｐＭＫＩＴＮｅｏ－Ｍｙｃタグで標識したＡＰＣ－ａｒｍは、ＡＰＣ（アミノ酸４４６－８８０）の部分配列をコードしているＤＮＡ断片をＳＲαプロモーターを有するｐＭＫＩＴＮｅｏに挿入することにより構築した。発現プラスミドは、リポフェクトアミン（ＬｉｐｏｆｅｃｔＡＭＩＮＥ；Ｌｉｆｅ　Ｔｅｃｈｎｏｌｏｇｉｅｓ社）を用いて、使用者マニュアルにしたがって、ＭＤＣＫ細胞にトランスフェクトした。

【００４５】ＭＤＣＫ細胞は、リン酸緩衝生理食塩水（Ｐｈｏｓｐｈａｔｅ　Ｂｕｆｆｅｒｅｄ　Ｓａｌｉｎｅ；ＰＢＳ）中で３．７％ホルムアルデヒドを用いて４℃で１時間、固定した。固定した細胞は室温で１０分間、０．２％トリトンＸ－１００を含むトリス緩衝生理食塩水（Ｔｒｉｓ　Ｂｕｆｆｅｒｅｄ　Ｓａｌｉｎｅ；ＴＢＳ）で処理し、ＴＢＳで３回洗浄した。細胞を透過化した（ｐｅｒｍｅａｂｉｌｉｚｅ）後、１％ＢＳＡ、３％ＦＢＳ、０．２％トリトンＸ－１００を含むＴＢＳ中で一次抗体と室温で１時間、インキュベートした。一次抗体を除去し、細胞をＴＢＳで３回洗浄した。結合した一次抗体は、ＦＩＴＣ（Ｆｌｕｏｒｅｓｃｅｉｎ　ｉｓｏｔｈｉｏｃｙａｎａｔｅ）またはローダミンを結合したヤギ二次抗体（Ｃａｐｐｅｌ社）を用いて、検出した。染色したサンプルは、カールツァイスＬＳＭ５１０レーザー走査顕微鏡（Ｃａｒｌ　Ｚｅｉｓｓ　ＬＳＭ５１０Ｌａｓｅｒ　ｓｃａｎｎｉｎｇ　ｍｉｃｒｏｓｃｏｐｅ）下で、観察した。

【００４６】結果は、図９に示す。図９中、ａ、ｂはＨＡ－タグで標識したＭ１蛋白質を発現させた同一細胞、ｃ、ｄはＨＡ－タグで標識したＭ１蛋白質とＭｙｃ－タグで標識したＡＰＣ－ａｒｍとを両方発現させた同一細胞、ｅはＨＡ－タグで標識したＭ１ΔＮＢ（アミノ酸１２７－６１９）を発現させた細胞、ｆはＭｙｃ－タグで標識したＡＰＣ－ａｒｍを発現させた細胞である。また、ａ、ｂは抗ＨＡ抗体と抗ＡＰＣ抗体で二重染色し、ｃ、ｄは抗ＨＡ抗体と抗Ｍｙｃ抗体で二重染色し、ｅは抗ＨＡ抗体で、ｆは抗Ｍｙｃ抗体で処理した。ｂ中の矢印頭で示すように、ＡＰＣ蛋白質のクラスターが伸長している膜中に存在しており、また、ａ～ｄ中の矢印で示すように、Ｍ１とＡＰＣが上皮細胞中で細胞の辺縁の同一部位に共存していることが判明した。

【００４７】（Ｍ１発現細胞株の形態解析）さらに、Ｍ１ΔＮＢを安定に発現するＭＤＣＫ細胞株を樹立し、Ｍ１が細胞の形態、骨格に与える影響を調べたところ、Ｍ１ΔＮＢ発現細胞株５クローンはいずれも密に接着せずばらばらに増殖する傾向が見られた。新規蛋白質Ｍ１の細胞接着への関与が推定された。

【００４８】

【発明の効果】以上説明したように本発明のＭ１は、新規蛋白質であり、そのＡＰＣ遺伝子産物のアルマジロリピート部位との結合性が特徴的で、ＧＥＦ（グアニンヌクレオチド交換因子：Ｇｕａｎｉｎｅ　ｎｕｃｌｅｏｔｉｄｅ　Ｅｘｃｈａｎｇｅ　Ｆａｃｔｏｒ）活性を有する。この特性を利用した新規医薬組成物、診療手段の提供は、ＡＰＣ遺伝子産物関連の臨床・基礎の医用領域において大きな有用性を提供する。

【配列表】

Sequence Listing

<110> Daiichi Pharmaceutical Co.,Ltd.

<120> Colon carcinoma suppressor gene related protein

<130> DP99-1045

<160> 2

<210> 1

<211> 619

<212> PRT

<213> human

<400> 1

Met Arg Pro Asp Gly Gln Gln Ala Leu Asp Ala Val Val Arg Ser Phe
1 5 10 15

Asp Cys His Ser Glu Ala Ala Leu Arg Gln Arg Asn Asp Val Ile Tyr
20 25 30

Cys Ser Leu Pro Arg Thr Ala Gln Gly Ile Val Gln Arg Glu Asp Gln
35 40 45

Leu Glu Val Leu Val Ser Leu Arg Glu Val Trp Gly Arg Arg Arg Gly
50 55 60

Arg Asp Gly Thr Cys Thr Gly Ala Met Pro Asp Gly Ala Leu Asp Thr
65 70 75 80

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Ala Val Cys Ala Asp Glu Val Gly Ser Glu Glu Asp Leu Tyr Asp Asp
                85                  90                  95
Leu His Ser Ser Ser His His Tyr Ser His Pro Gly Gly Gly Gly Glu
            100                 105                 110
Gln Leu Ala Ile Asn Glu Leu Ile Ser Asp Gly Ser Val Val Cys Ala
        115                 120                 125
Glu Ala Leu Trp Asp His Val Thr Met Asp Asp Gln Glu Leu Gly Phe
    130                 135                 140
Lys Ala Gly Asp Val Ile Glu Val Met Asp Ala Thr Asn Arg Glu Trp
145                 150                 155                 160
Trp Trp Gly Arg Val Ala Asp Gly Glu Gly Trp Phe Pro Ala Ser Phe
                165                 170                 175
Val Arg Leu Arg Val Asn Gln Asp Glu Pro Ala Asp Asp Asp Ala Pro
            180                 185                 190
Leu Ala Gly Asn Ser Gly Ala Glu Asp Gly Gly Ala Glu Ala Gln Ser
        195                 200                 205
Ser Lys Asp Gln Met Arg Thr Asn Val Ile Asn Glu Ile Leu Ser Thr
    210                 215                 220

Glu Arg Asp Tyr Ile Lys His Leu Arg Asp Ile Cys Glu Gly Tyr Val
225                 230                 235                 240
Arg Gln Cys Arg Lys Arg Ala Asp Met Phe Ser Glu Glu Gln Leu Arg
                245                 250                 255
Thr Ile Phe Gly Asn Ile Glu Asp Ile Tyr Arg Cys Gln Lys Ala Phe
            260                 265                 270
Val Lys Ala Leu Glu Gln Arg Phe Asn Arg Glu Arg Pro His Leu Ser
        275                 280                 285
Glu Leu Gly Ala Cys Phe Leu Glu His Gln Ala Asp Phe Gln Ile Tyr
    290                 295                 300
Ser Glu Tyr Cys Asn Asn His Pro Asn Ala Cys Val Glu Leu Ser Arg
305                 310                 315                 320
Leu Thr Lys Leu Ser Lys Tyr Val Tyr Phe Phe Glu Ala Cys Arg Leu
                325                 330                 335
Leu Gln Lys Met Ile Asp Ile Ser Leu Asp Gly Phe Leu Leu Thr Pro
            340                 345                 350
Val Gln Lys Ile Cys Lys Tyr Pro Leu Gln Leu Ala Glu Leu Leu Lys
        355                 360                 365
Tyr Thr His Pro Gln His Arg Asp Phe Lys Asp Val Glu Ala Ala Leu
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Leu Glu Asn Ile Asp Lys Ile Ala Gln Trp Gln Ser Ser Ile Glu Asp
                405                 410                 415
Trp Glu Gly Glu Asp Leu Leu Val Arg Ser Ser Glu Leu Ile Tyr Ser
            420                 425                 430
Gly Glu Leu Thr Arg Val Thr Gln Pro Gln Ala Lys Ser Gln Gln Arg
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Met Phe Phe Leu Phe Asp His Gln Leu Ile Tyr Cys Lys Lys Asp Leu
    450                 455                 460
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465                 470                 475                 480
Leu Glu Val Val Asp Leu Glu Asp Gly Lys Asp Arg Asp Leu His Val
                485                 490                 495
Ser Ile Lys Asn Ala Phe Arg Leu His Arg Gly Ala Thr Gly Asp Ser
            500                 505                 510
His Leu Leu Cys Thr Arg Lys Pro Glu Gln Lys Gln Arg Trp Leu Lys
        515                 520                 525
Ala Phe Ala Arg Glu Arg Glu Gln Val Gln Leu Asp Gln Glu Thr Gly
    530                 535                 540
Phe Ser Ile Thr Glu Leu Gln Arg Lys Gln Ala Met Leu Asn Ala Ser
545                 550                 555                 560
Lys Gln Gln Val Thr Gly Lys Pro Lys Ala Val Gly Arg Pro Cys Tyr
                565                 570                 575
Leu Thr Arg Gln Lys His Pro Ala Leu Pro Ser Asn Arg Pro Gln Gln
            580                 585                 590
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gac tgt cac tct gaa gca gct ctc agg cag agg aat gat gtg att tat      96
Asp Cys His Ser Glu Ala Ala Leu Arg Gln Arg Asn Asp Val Ile Tyr
            20                  25                  30
tgt agt tta cca agg acc gct cag ggt att gtg cag agg gaa gac cag     144
Cys Ser Leu Pro Arg Thr Ala Gln Gly Ile Val Gln Arg Glu Asp Gln
        35                  40                  45
ctg gag gtt ctt gtg tca ctc cgt gaa gtg tgg ggg cgg cgg agg ggc     192
Leu Glu Val Leu Val Ser Leu Arg Glu Val Trp Gly Arg Arg Arg Gly
    50                  55                  60
aga gat ggg acc tgc act gga gcc atg cct gat gga gct ctg gac aca     240
Arg Asp Gly Thr Cys Thr Gly Ala Met Pro Asp Gly Ala Leu Asp Thr
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Ala Val Cys Ala Asp Glu Val Gly Ser Glu Glu Asp Leu Tyr Asp Asp
                85                  90                  95
ctg cac agc tcc agc cac cac tac agc cac cct gga ggg ggt ggg gag     336
Leu His Ser Ser Ser His His Tyr Ser His Pro Gly Gly Gly Gly Glu
            100                 105                 110
cag ctg gct atc aat gag ctc atc agc gat ggc agt gtg gtc tgc gct     384
Gln Leu Ala Ile Asn Glu Leu Ile Ser Asp Gly Ser Val Val Cys Ala
        115                 120                 125
gaa gca ctc tgg gac cat gtc acc atg gac gac cag gag ctg ggc ttc     432
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Glu Ala Leu Trp Asp His Val Thr Met Asp Asp Gln Glu Leu Gly Phe
    130                 135                 140

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Lys Ala Gly Asp Val Ile Glu Val Met Asp Ala Thr Asn Arg Glu Trp
145                 150                 155                 160
tgg tgg ggc cgg gtc gcc gat ggc gag ggc tgg ttt cca gcc agc ttc     528
Trp Trp Gly Arg Val Ala Asp Gly Glu Gly Trp Phe Pro Ala Ser Phe
                165                 170                 175
gtt cgg ctg agg gtg aat cag gac gag ccc gcg gat gac gac gcc cct     576
Val Arg Leu Arg Val Asn Gln Asp Glu Pro Ala Asp Asp Asp Ala Pro
            180                 185                 190
ctg gcc ggg aac agc gga gcg gag gac ggc ggg gcg gag gcg cag agc     624
Leu Ala Gly Asn Ser Gly Ala Glu Asp Gly Gly Ala Glu Ala Gln Ser
        195                 200                 205
agc aag gac cag atg cgg acc aac gtc atc aac gag atc ctc agc act     672
Ser Lys Asp Gln Met Arg Thr Asn Val Ile Asn Glu Ile Leu Ser Thr
    210                 215                 220
gag cgg gac tac atc aag cac ctg cgc gac atc tgc gag ggc tac gtc     720
Glu Arg Asp Tyr Ile Lys His Leu Arg Asp Ile Cys Glu Gly Tyr Val
225                 230                 235                 240
cgg cag tgc cgc aag cgc gca gac atg ttc agc gag gag cag ctg cgt     768
Arg Gln Cys Arg Lys Arg Ala Asp Met Phe Ser Glu Glu Gln Leu Arg
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acc atc ttc ggg aac atc gag gac atc tac cgc tgc cag aag gcc ttc     816
Thr Ile Phe Gly Asn Ile Glu Asp Ile Tyr Arg Cys Gln Lys Ala Phe
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Val Lys Ala Leu Glu Gln Arg Phe Asn Arg Glu Arg Pro His Leu Ser
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gag ctg ggt gcc tgc ttc ctg gag cat caa gcc gac ttc cag atc tac     912
Glu Leu Gly Ala Cys Phe Leu Glu His Gln Ala Asp Phe Gln Ile Tyr
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tcg gag tac tgc aat aac cac ccc aac gcc tgc gtg gag ctc tcc cgg     960
Ser Glu Tyr Cys Asn Asn His Pro Asn Ala Cys Val Glu Leu Ser Arg
305                 310                 315                 320
ctc acc aag ctc agc aag tac gtg tac ttc ttc gag gcc tgc cgg ctg    1008
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        355                 360                 365
tac acg cac ccc cag cac agg gac ttc aag gat gtt gaa gcc gcc ttg    1152
Tyr Thr His Pro Gln His Arg Asp Phe Lys Asp Val Glu Ala Ala Leu
    370                 375                 380
cat gcc atg aag aac gtg gcc cag ctc atc aac gag cgg aag cgg aga    1200
```

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His Ala Met Lys Asn Val Ala Gln Leu Ile Asn Glu Arg Lys Arg Arg
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                405                 410                 415
tgg gag gga gaa gat ctc ttg gtc agg agc tca gaa ctc atc tac tcg    1296
Trp Glu Gly Glu Asp Leu Leu Val Arg Ser Ser Glu Leu Ile Tyr Ser
            420                 425                 430
ggg gag ctg act cga gtt aca cag cct caa gcc aaa agc cag cag cga    1344
Gly Glu Leu Thr Arg Val Thr Gln Pro Gln Ala Lys Ser Gln Gln Arg
        435                 440                 445
atg ttc ttt ctc ttt gac cac cag ctc atc tac tgt aag aag gac ctg    1392
Met Phe Phe Leu Phe Asp His Gln Leu Ile Tyr Cys Lys Lys Asp Leu
    450                 455                 460
ctc cgc cgc gac gtg ttg tac tac aag ggc cgg ctg gac atg gac ggc    1440
Leu Arg Arg Asp Val Leu Tyr Tyr Lys Gly Arg Leu Asp Met Asp Gly
465                 470                 475                 480
ctg gag gtg gtg gac ctg gag gac ggg aag gac aga gac ctc cat gtg    1488
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Ser Ile Lys Asn Ala Phe Arg Leu His Arg Gly Ala Thr Gly Asp Ser
            500                 505                 510
cac ctg ctg tgc acc agg aag ccc gag cag aag cag cgc tgg ctc aag    1584
His Leu Leu Cys Thr Arg Lys Pro Glu Gln Lys Gln Arg Trp Leu Lys
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gcc ttt gcc agg gag agg gag cag gtg cag ctg gac cag gag aca ggc    1632
Ala Phe Ala Arg Glu Arg Glu Gln Val Gln Leu Asp Gln Glu Thr Gly
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545                 550                 555                 560
aag cag cag gtc aca ggg aag ccc aaa gct gtt ggc cgg ccc tgc tac    1728
Lys Gln Gln Val Thr Gly Lys Pro Lys Ala Val Gly Arg Pro Cys Tyr
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ctg acg cgc cag aag cac cca gcc ctg ccc agc aac cgg ccc cag cag    1776
Leu Thr Arg Gln Lys His Pro Ala Leu Pro Ser Asn Arg Pro Gln Gln
            580                 585                 590
cag gtc ctg gtg ctg gcg gag ccc agg cgc aag cca tct acc ttc tgg    1824
Gln Val Leu Val Leu Ala Glu Pro Arg Arg Lys Pro Ser Thr Phe Trp
        595                 600                 605

cac agc atc agc cgg ctg gca ccc ttc cgc aag tga                   1860
His Ser Ile Ser Arg Leu Ala Pro Phe Arg Lys *
    610                 615                 620
```

【図面の簡単な説明】

【図１】　新規蛋白質Ｍ１のアミノ酸配列の特徴および相同ドメインの配列比較を説明する図である。（Ａ）ヒトＭ１の推定アミノ酸配列。ＳＨ３ドメインは太字、ＤＨドメインは囲み太字、ＰＨドメインは太字かつ下線で表示する。（Ｂ）Ｍ１と、Ｄｂｌファミリーのサブファミリーメンバー：ＫＩＡＡ０４２４、Ｄｂｌ、Ｔｉａｍ－１、Ｖａｖ、ｐ１１５－ＲｈｏＧＥＦ、ＣＤＣ２４およびＳｏｓ１との比較。ＤＨドメインの相同性はＭ１のＤＨドメインとほかのタンパクのＤＨドメインとの対応

比較における同一性／相同性の割合として計算した。

（C）DHドメインのアミノ酸配列表。M1、KIAA0424、Dbl、Tiam−1、Vav、p115−RhoGEF、CDC24およびSos1のDHドメインを並列し、さらに最適化した。同一アミノ酸は白抜き文字で示す。（D）PHドメインのアミノ酸配列表。M1と他のGEF蛋白質および基準となるヒトプレクストリン（pleckstrin）に存在するPHドメインを並列した。同一アミノ酸は白抜き文字で示す。

【図2】　新規蛋白質M1の発現を、ラット胎児脳、およびM1cDNAを組み込んだ発現プラスミドpcDNA3．1（+）をトランスフェクトしたCOS−7細胞で確認した図面である。図中、レーン1、2はラット胎児脳、レーン3〜9は形質転換したCOS−7細胞でのM1発現を示し、形質転換のためのベクターとして、レーン3と4はM1cDNAを組み込んだベクター、レーン5はコントロールベクター、レーン6と8はHA−タグで標識したM1cDNAを組み込んだベクター、レーン7と9はHA−タグを組み込んだコントロールベクターを使用した。また、レーン1、3、5は、予め抗M1抗体を対応する抗原であるM1ペプチドで吸収して（pep．+）用いた結果である。

【図3】　新規蛋白質M1とAPC遺伝子産物とのin vivoでの結合をラット胎児脳溶解物について解析した結果を示す図面である。図中、レーン1および2はラット胎児脳の溶解物を抗M1抗体で、レーン3および4は抗APC抗体で、レーン5および6は抗β−カテニン抗体で免疫沈降した結果、レーン1、3、5は、予め各抗体を対応する抗原で吸収して（pep．+）用いた結果である。

【図4】　新規蛋白質M1とAPC遺伝子産物との結合部位を説明する図である。+はAPC遺伝子産物との結合活性陽性、−は陰性を表す。＊はAPC遺伝子産物のアルマジロ配列をベイトとした2ハイブリッドスクリーニングで得られたクローンを示す。

【図5】　新規蛋白質M1とRhoファミリー低分子量G蛋白質との結合を示す図面である。

【図6】　新規蛋白質M1の低分子量G蛋白質からのGDP解離に対する促進作用を示す図であり、図Aは低分子量G蛋白質Rac1に対する作用、図Bは低分子量G蛋白質RhoAに対する作用を示す。。

【図7】　新規蛋白質M1の低分子量G蛋白質へのGTP結合に対する促進作用を示す図であり、図Aは低分子量G蛋白質Rac1に対する作用、図Bは低分子量G蛋白質RhoAに対する作用を示す

【図8】　新規蛋白質M1のGEF活性が、APC−アルマジロドメインの添加により増強されることを示す図である。

【図9】　新規蛋白質M1とAPC遺伝子産物とが、それらを発現した上皮細胞株中の細胞の辺縁の同一部位に存在することを示す図面である。図中、a、bはHA−タグで標識したM1蛋白質を発現させた同一細胞、c、dはHA−タグで標識したM1蛋白質とMyc−タグで標識したAPC−armとを両方発現させた同一細胞、eはHA−タグで標識したM1△NB（アミノ酸127−619）を発現させた細胞、fはMyc−タグで標識したAPC−armを発現させた細胞である。また、a、bは抗HA抗体と抗APC抗体で二重染色し、c、dは抗HA抗体と抗Myc抗体で二重染色し、eは抗HA抗体で、fは抗Myc抗体で処理したものである。b中の矢印頭は、APC蛋白質のクラスターの存在を示しており、また、a〜d中の矢印は、M1とAPCの存在を示す。

【図1】

A

MRPDGQQALDAVVRSFDCHSEAALRQRNDVIYCSLPRTAQGIVQREDQL
EVLVSLREVWGRRRGRDGTCTGAMPDGALDTAVCADEVGSEEDLYDDLH
SSSHHYSHPGGGGEQLAINELISDGSVVCAEALWDHVTMDDQELGFKAG
DVIEVMDATNREWWWGRVADGEGWFPASFVRLRVNQDEPADDDAPLAGN
SGAEDGGAEAQSSKDQMRTNVINEILSTERDYIKHLRDICEGYVRQCRK
RADMFSEEQLRTIFGNIEDIYRCQKAFVKALEQRFNRERPHLSELGACF
LEHQADFQIYSEYCNNHPNACVELSRLTKLSKYVYFFEACRLLQKMIDI
SLDGFLLTPVQKICKYPLQLAELLKYTHPQHRDFKDVEAALHAMKNVAQ
LINERKRRLENIDKIAQWQSSIEDWEGEDLLVRSSELIYSGELTRVTQP
QAKSQQRMFFLFDHQLIYCKKDLLRRDVLYYKGRLDMDGLEVVDLEDGK
DRDLHVSIKNAFRLHRGATGDSHLLCTRKPEQKQRWLKAFARELEQVQL
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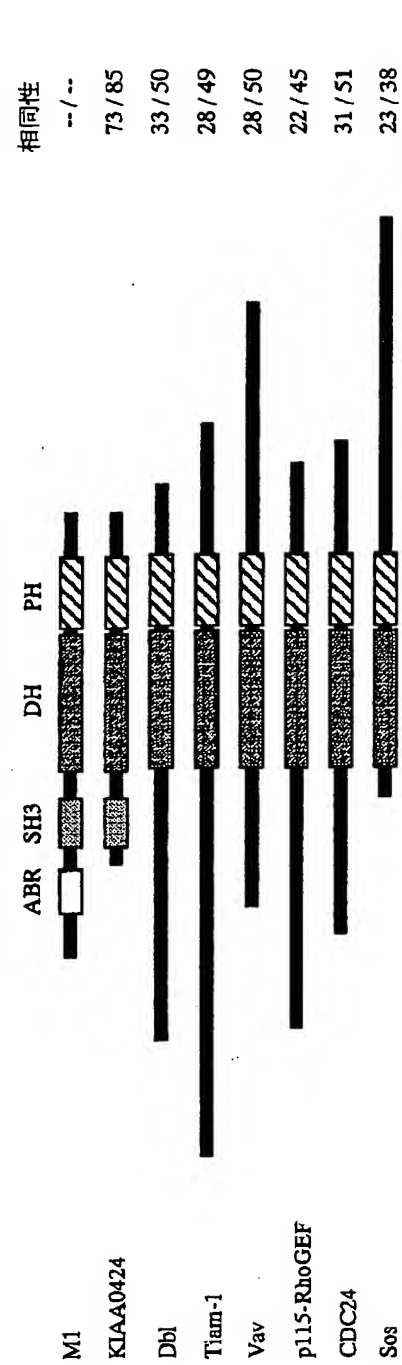
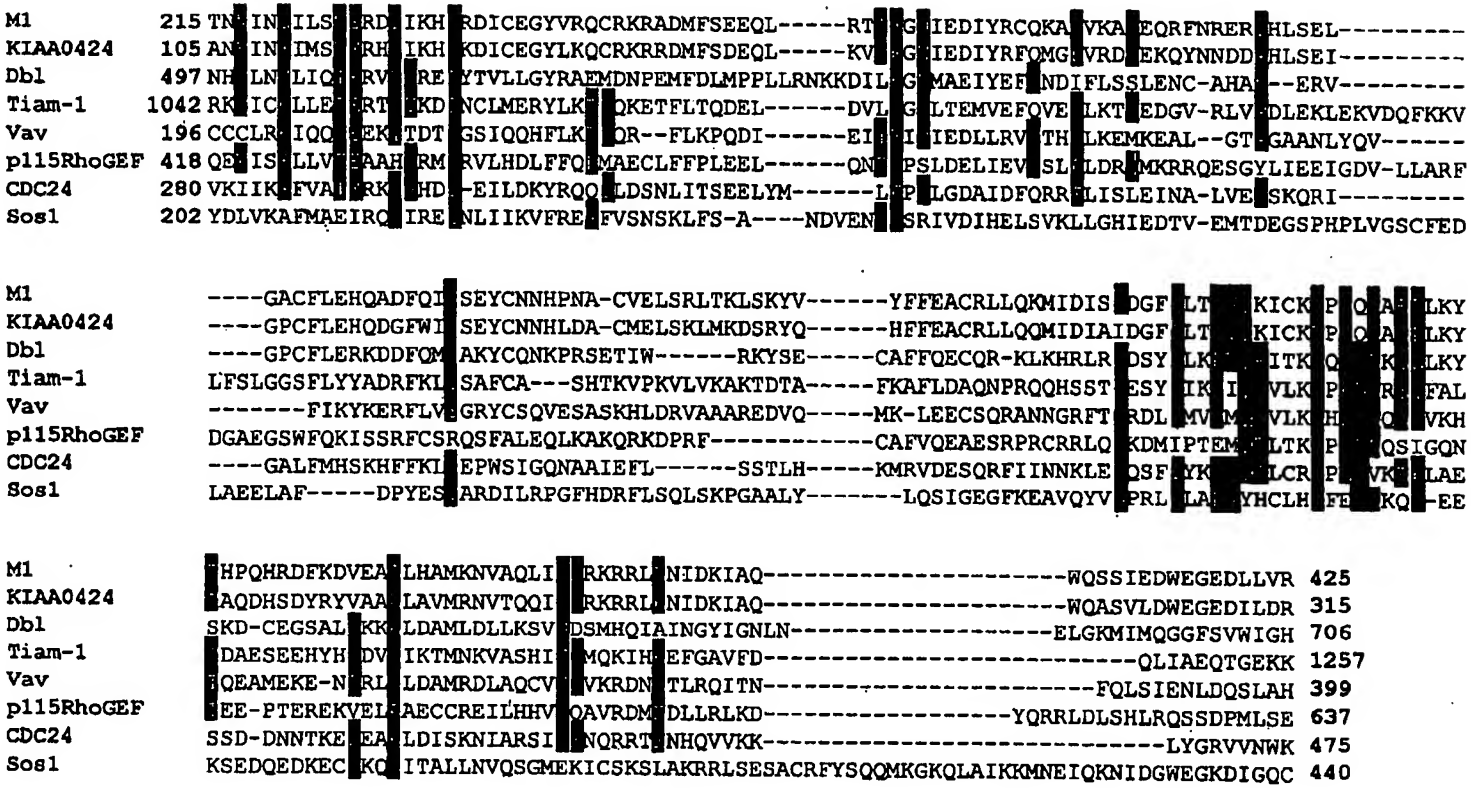
RPQQQVLVLAEPRRKPSTFWHSISRLAPFRK*

B


DH ドメイン
相同性
ABR
SH3
DH
PH
M1
--/--
KIAA0424
73/85
Dbl
33/50
Tiam-1
28/49
Vav
28/50
p115-RhoGEF
22/45
CDC24
31/51
Sos
23/38

C



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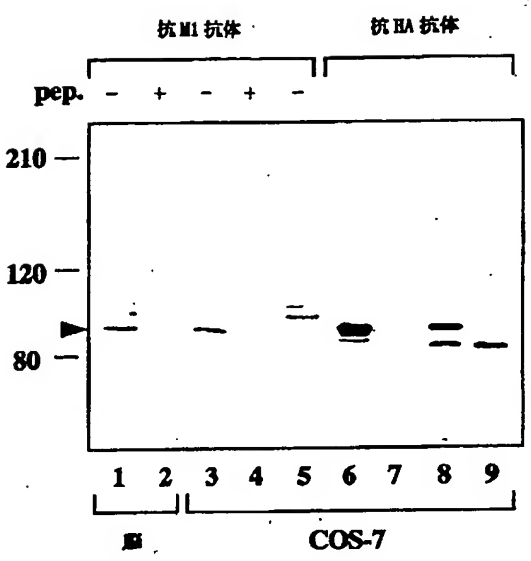
【図２】



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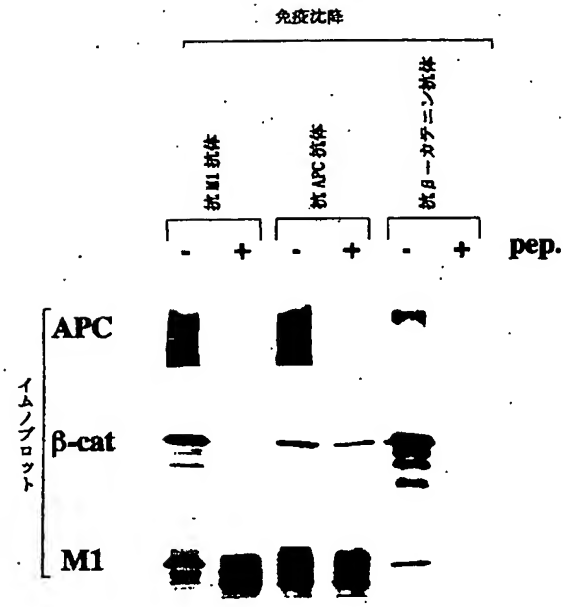

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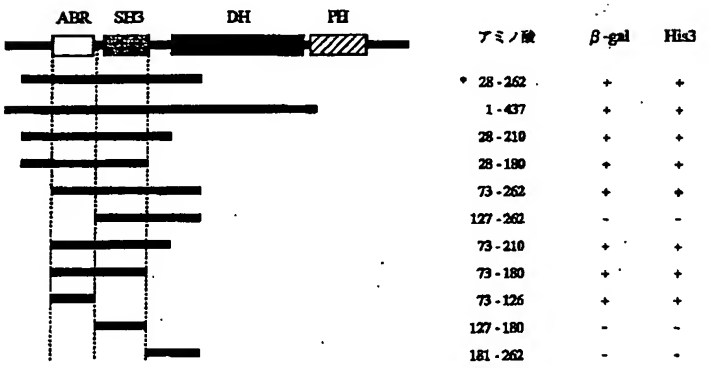

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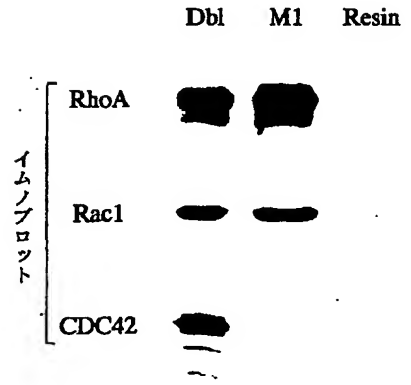

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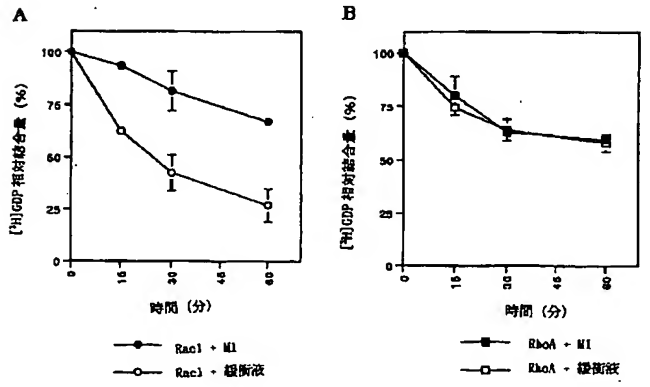

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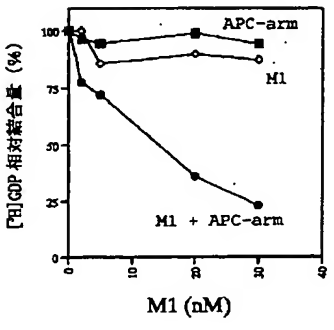

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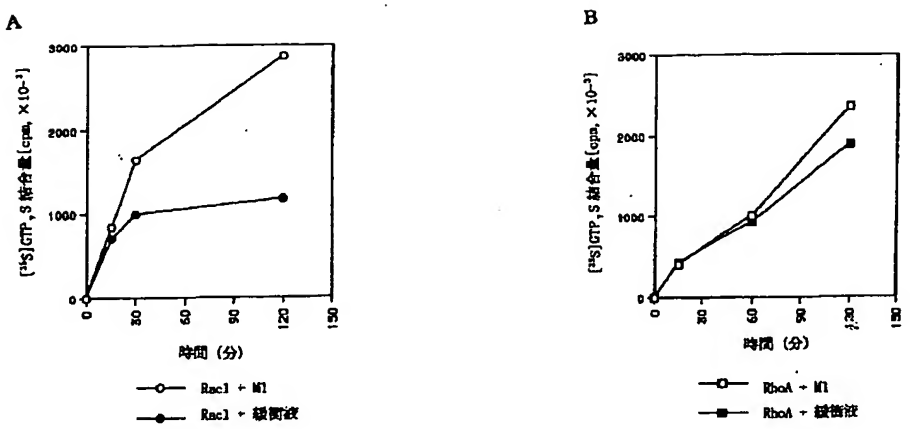

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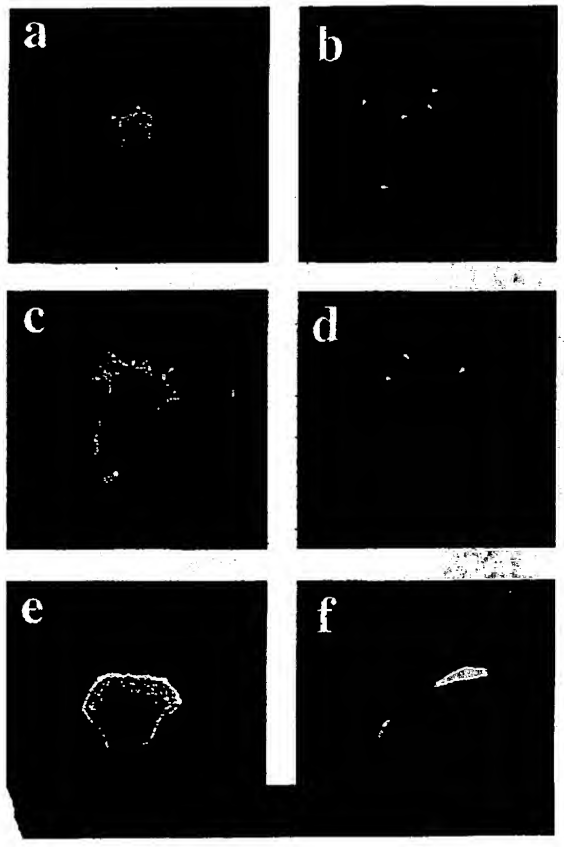

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	16/18	
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	5/10	
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Ｇ０１Ｎ	33/15	
	33/50	
	33/574	
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	16/18	4Ｈ０４５
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4C084 AA02 AA06 AA07 AA13 AA24
BA01 BA08 BA22 CA27 CA53
DA39 DC50 MA01 NA14 ZB262
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C12N 1/15
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(71)Applicant : DAI ICHI SEIYAKU CO LTD

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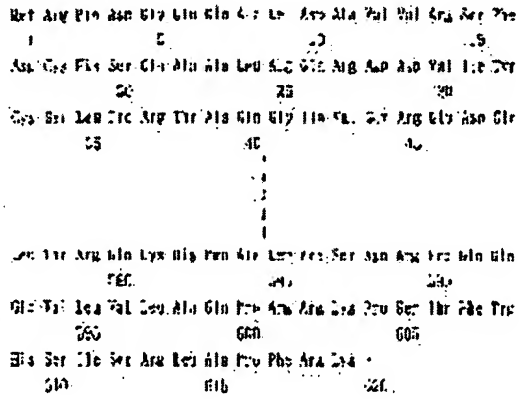
KAWASAKI YOSHIHIRO

(54) COLON CARCINOMA SUPPRESSOR GENE-RELATED PROTEIN

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a new protein which has a specific amino acid sequence, has an ability of binding an armadillo repeat site of a gene product of a colon carcinoma suppressor gene, can control the colon carcinoma, and is useful for treating and diagnosing the colon tumor and so on.

SOLUTION: This is a new colon carcinoma suppressor gene-related protein which is a polypeptide selected from a group comprising a polypeptide having the amino acid sequence shown by the formula, polypeptides containing the amino acid sequence shown by the



formula, polypeptides having a homology of about 70% or more to a polypeptide having the amino acid sequence shown by the formula and an ability of binding a polypeptide encoded by the armadillo repeat site of a gene product of a colon carcinoma suppressor gene (Adenomatous Polyposis Coli, APC), and the like, allows the control of APC, and is useful for treating and/or diagnosing the colon carcinoma and so on. This protein is obtained by identifying cDNA of a protein binding an APC gene product from a fetal brain cDNA library for expression.

LEGAL STATUS

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CLAIMS

[Claim(s)]
[Claim 1] Polypeptide chosen from the following group;
** The polypeptide shown in the array number 1 of an array table according to the amino acid sequence of a publication, ** The polypeptide containing the amino acid sequence of the polypeptide of the aforementioned **, It has the homology on about 70% of amino acid sequence at least with the polypeptide of the aforementioned **. ** And the armadillo repeat part of the gene product of the antioncogene (Adenomatous Polyposis Coli:APC) of colon cancer In the amino acid sequence of the polypeptide which has a binding affinity to the polypeptide which carries out a code, and the polypeptide of the ** aforementioned ** to ** 1 thru/or the deletion of some amino acid, The polypeptide which has variation or induced mutation, such as a permutation and addition, and has a binding affinity to the armadillo repeat part of an APC gene product.
[Claim 2] The polypeptide which has at least five amino acid sequences of the amino acid sequence of a publication for the array number 1 of an array table, and has a binding affinity to the armadillo repeat part of an APC gene product.
[Claim 3] The polynucleotide which carries out the code of the polypeptide according to claim 1 or 2, or its complementary strand.
[Claim 4] The polynucleotide which carries out hybridization to a polynucleotide or its complementary strand according to claim 3 under stringent conditions.
[Claim 5] The polynucleotide in which it is the polynucleotide shown in the array number 2 of an array table according to at least 15 continuous base sequences among the polynucleotide of a publication, or the base sequence of the complementary strand, and the polypeptide discovered by the imprint of this polynucleotide has a binding affinity to the armadillo repeat part of an APC gene product.
[Claim 6] The recombination vector which contains the polynucleotide of a publication in any 1 term of claims 3-5.
[Claim 7] The transformant by which the transformation was carried out by the recombination vector of claim 6.
[Claim 8] The manufacture approach including the process which cultivates the transformant of claim 7 of a polypeptide according to claim 1 or 2.
[Claim 9] The antibody which recognizes immunologically a polypeptide according to claim 1 or 2.
[Claim 10] The screening approach which is the screening approach of the compound which checks or reinforces the affinity over the armadillo repeat part of an APC gene product of a polypeptide according to claim 1, and is characterized by using any one at least among a polypeptide according to claim 1 or 2 and an antibody according to claim 9.
[Claim 11] The screening approach which is the screening approach of the compound which interacts with the polynucleotide of a publication to claim 3 or 4, and checks or reinforces the manifestation of this polynucleotide to it, and is characterized by using any one for any 1 term of claims 3-5 at least among the polynucleotide of a publication, a vector according to claim 6, a transformant according to claim 7, and an antibody according to claim 9.
[Claim 12] The screening approach which is the screening approach of the compound which checks or reinforces the GEF (guanine-nucleotide exchange factor: Guanine nucleotide ExchangeFactor) activity of a polypeptide according to claim 1, and is characterized by using any one at least among a polypeptide according to claim 1 or 2 and an antibody according to claim 9.
[Claim 13] The compound screened by any 1 term of claims 10-12 by the screening approach of a publication.
[Claim 14] The compound which checks or reinforces the affinity over the armadillo repeat part of an APC gene product of a polypeptide according to claim 1.
[Claim 15] The compound which interacts with the polynucleotide of a publication in any 1 term of claims 3-5, and checks or reinforces the manifestation of this polynucleotide in it.
[Claim 16] The compound which checks or reinforces the GEF activity of a polypeptide according to claim 1.

[Claim 17] The physic constituent which is characterized by containing any one at least among the compounds of a publication in any 1 term of a polype[redacted]e according to claim 1 or 2, a polynucleo[redacted]tide given in any 1 term of claims 3-5, a vector according to claim 6, a tra[redacted]rmant according to claim 7, an antibod[redacted]ording to claim 9, or claims 13-16 and which is used for the therapy of a large intestine neoplasm.

[Claim 18] The polynucleotide which is the manifestation of the polypeptide of claim 1, or the diagnostic means of the illness relevant to activity, and is carrying out the code of the (a) this polypeptide in a sample, and/or a diagnostic means including analyzing (b) this polypeptide as a marker.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the new protein (M1 is called hereafter) and the new polypeptide which have a binding affinity to the polypeptide in which the antioncogene (Adenomatous Polyposis Coli:APC) which participates in colon cancer carries out a code, especially a binding affinity to the armadillo repeat part of an APC gene product. The polypeptide which has all or a part of amino acid sequences of the new protein M1 in more detail, The recombination vector containing the polynucleotide which carries out the code of this polypeptide, and this polynucleotide, The manufacture approach of of the peptide or polypeptide using the transformant and this transformant by which the transformation was carried out by this recombination vector, The antibody to this peptide or a polypeptide, the screening approach of a compound of having used these, It is related to the activity inhibition compound which acts on the screened this compound, this polypeptide, or this polynucleotide or an activity activation compound, the physic constituent related to these, and the illness diagnostic means related to these.
[0002]
[Description of the Prior Art] Antioncogene APC is isolated as a gene of cause of the familial adenoma nature polyposis (familial adenomatous polyposis:FAP), and the abnormalities of this APC are reported according to 70 - 80% of the colon cancer of sporadicness. An APC gene product is huge protein of 300kDa(s) which consists of 2,843 amino acid (170 Cell, 87:159- 1996). The interaction with various protein is known and an APC gene product has beta-KATENIN in one of them. beta-KATENIN is functioning also as one of the important components of the Wnt/Wingless signal transfer path which bears an important role in a generating process or tumorigenesis at the same time it combines with the cytoplasm side domain of cadherin and it plays a role in cell adhesion (642 Nature, 382:638- 1996). (399 Cell, 86:391- 1996) beta-KATENIN is a kind of oncogene product, and it is thought that the APC gene product demonstrates the cancer control function by controlling the function of beta-KATENIN (1792 Science, 275:1790- 1997). (1787 Science, 275:1784- 1997) (1790 Science, 275:1787- 1997) In addition, as for the APC gene product, the interaction with GSK-3b, Axin, or KONDA cutin / Axil is known (403 Genes Cells, 6:395- 1998). (599 Science, 280:596- 1998) (581 Current Biology, 8:573- 1998) (10826 J. Biol.Chem., 273:10823- 1998) Moreover, the interaction the APC gene product minded the C terminal of EB1 and hDLG is also reported (1023 Science, 272:1020- 1996). Furthermore, it is also known that the armadillo repeat domain which plays a role of the interaction between protein exists in an APC gene product.
[0003]
[Problem(s) to be Solved by the Invention] The technical problem which this invention tends to solve is finding out the new matter which participates in the APC gene which is an antioncogene of the colon cancer which bears an interaction with matter various as mentioned above, and is using this new matter as a means aiming at control of cancer. The technical problem of this invention is offering the new matter (M1) with a binding affinity with an APC gene product, especially a binding affinity with the

armadillo repeat domain of the APC gene product origin, and, more specifically, is offering the polypeptide of the useful new matter (M1) origin in connection with it. Moreover, another technical problem of this invention is offering the polynucleotide which carries out the code of the polypeptide of the M1 origin, and offering the manufacturing method of the polypeptide of the M1 origin by the gene engineering technique. Technical problem that this invention is still more nearly another is offering the antibody to the polypeptide of the M1 origin. The technical problem of other this inventions is screening the inhibitor, antagonist, and activator of the operation which M's1 has using the above-mentioned thing, and is offering the screened compound, and is offering the physic constituent and diagnostic means used for the large intestine neoplasm using these.

[0004]

[Means for Solution] For technical-problem solution, this invention person identified cDNA of the new protein M1 combined with the armadillo repeat domain (arm may be called hereafter) of an APC gene product using 2 hybrid screening procedure from the Homo sapiens embryo brain cDNA library, determined the amino acid sequence in which cDNA of the base sequence and this new protein M1 carries out a code, and completed this invention.

[0005] Namely, the polypeptide this invention is indicated to be to the array number 1 of the polypeptide;** array table chosen from the following group according to the amino acid sequence of a publication, ** The polypeptide containing the amino acid sequence of the polypeptide of the aforementioned **, It has the homology on about 70% of amino acid sequence at least with the polypeptide of the aforementioned **. ** And the armadillo repeat part of the gene product of the antioncogene (Adenomatous Polyposis Coli:APC) of colon cancer In the amino acid sequence of the polypeptide which has a binding affinity to the polypeptide which carries out a code, and the polypeptide of the ** aforementioned ** to ** 1 thru/or the deletion of some amino acid, The polypeptide which has variation or induced mutation, such as a permutation and addition, and has a binding affinity to the armadillo repeat part of an APC gene product; It has at least five amino acid sequences of the amino acid sequence of a publication for the array number 1 of an array table. To and the armadillo repeat part of an APC gene product The receiving binding affinity The polypeptide which it has; the polypeptide of this invention The polynucleotide which carries out a code Or the complementary strand; The polynucleotide of this invention Or the polynucleotide which carries out hybridization to the complementary strand under stringent conditions; It is the polynucleotide shown according to at least 15 continuous base sequences among the polynucleotide of this invention, or the base sequence of the complementary strand. The polypeptide discovered by the imprint of this polynucleotide has a binding affinity to the armadillo repeat part of an APC gene product. polynucleotide; -- recombination vector; containing the polynucleotide of this invention -- the process which cultivates the transformant of transformant; this invention by which the transformation was carried out by the recombination vector of this invention is included -- The manufacture approach of the polypeptide of this invention; It is the screening approach of the compound which checks or reinforces the affinity over the armadillo repeat part of the APC gene product of the polypeptide of this invention. The screening approach characterized by using any one at least among the polypeptide of this invention, and the antibody of this invention; It is the screening approach of the compound which interacts with the polynucleotide of this invention, and checks or reinforces the manifestation of this polynucleotide. The polynucleotide of this invention, the vector of this invention, the transformant of this invention, Among the antibodies of this invention, at least any one The screening approach characterized by using; It is the screening approach of the compound which checks or reinforces the GEF (guanine-nucleotide exchange factor: Guanine nucleotide Exchange Factor) activity of the polypeptide of this invention. The polypeptide of this invention, Among the antibodies of this invention, at least any one Using It interacts with the polynucleotide of compound; this invention which checks or reinforces the affinity over the armadillo repeat part of the APC gene product of the polypeptide of compound; this invention screened by the screening approach of this invention. screening approach; by which it is characterized -- The compound which checks or reinforces the GEF activity of the polypeptide of compound; this invention which checks or reinforces the manifestation of this polynucleotide; The polypeptide of this invention,

The polynucleotide of this invention, the vector of this invention, the transformant of this invention, The physic constituent used for the therapy of the large intestine neoplasm characterized by containing any one at least among the antibody of this invention, or the compound of this invention; They are the manifestation of the polypeptide of this invention, or the diagnostic means of the illness relevant to activity. The polynucleotide which is carrying out the code of the (a) this polypeptide in a sample, and/or a diagnostic means including analyzing (b) this polypeptide as a marker are offered.

[0006]

[Embodiment of the Invention] (New M1) The cDNA is acquired as matter to which the polynucleotide which carries out the code of the new protein M1 offered in this invention uses the armadillo repeat domain of an APC gene product for, and has a new amino acid sequence by 2 hybrid screening procedure from a human embryo brain cDNA library. cDNA of the new protein M1 of this invention carried out the code of the protein which consists of 619 amino acid, and had domain structure similar to a known Dbl family (it is one of the GDP dissociation promotion protein which acts on a low-molecular-weight G protein Rho family) (222 Current Opinion in CellBiology, 8:216- 1996). When the gene database (The National Center for Biotechnology Information) was searched, it became clear that cDNA of the new protein M1 of this invention had the known matter KIAA0424 and about 73% of homology who are one of the Dbl families. KIAA0424 -- the new protein M1 of this invention -- this -- it has the biggest difference at the point lacked in the N terminal region of M1. Both are the same in the point which supports a Dbl homologous (DH) domain, a PUREKKUSUTORIN (Preckstrin) homologous (PH) domain, and Src homologous 3 (SH3) domain. moreover, organization distribution research of the new protein M1 using a mouse -- setting -- this -- it checked that mRNA of M1 was discovered with a high level in the brain, and had discovered other organs by the low.

[0007] The antibody produced using the peptide fragment (amino acid 73-126 of an array table and the array number 1) of the new protein M1 showed strong reactivity to the new protein M1 obtained as a fusion protein with a glutathione-S-transferase (GST). Moreover, the new matter M1 which exists in a brain checked that it was the protein of about 85 kDa(s) by the system of measurement using an antigen-antibody reaction using the produced this antibody.

[0008] Moreover, it sets to the experiment for checking the direct interaction of the new protein M1 and an APC gene product. Although the armadillo repeat domain (APC-arm) of the APC gene product acquired as a fusion protein with a glutathione-S-transferase (GST) interacted with GST fusion M1 fragment (GST-M1-M) With a GST independent, although it did not react but M1-M reacted with GST-APC-arm similarly, it checked not reacting with the armadillo repeat domain of GST fusion beta-KATENIN, or a GST independent. That is, it is presumed that the new protein M1 is combined through the armadillo repeat domain of an APC gene product and this APC gene product. Moreover, it became clear by carrying out immunoprecipitation of the melt (lysate) of a rat embryo brain by the anti-APC antibody, and subsequently carrying out an immuno blot by anti-M1 antibody that M1 of this invention acted to an APC gene product as Mr. [coprecipitation] In this reaction, when anti-M1 antibody was pretreated in M1 fragment holding antigenic, the coprecipitation of M1 and an APC gene product was checked. That is, M1 and an APC gene product are considered to have joined together in the living body. Moreover, when the bonding site of M1 and an APC gene product was checked using 2 hybrid methods, it was presumed that a bonding site exists in the field shown from the amino acid 73-126 of M1 at least. This means that a binding site with the armadillo repeat domain of an APC gene product exists in the upstream region of SH3 domain of M1. Since KIAA0424 does not have such a field, it does not have reactivity with an APC gene product.

[0009] Furthermore, it checked that the new protein M1 had specific GEF activity in Rac which is one of the Rho families as the operation. That is, it combines with Rac, and the new protein M1 promotes GDP/GTP exchange reaction, activates Rac, and acts on NFkappaB, c-jun, SRE, etc. which are located in the lower stream of a river of cell signal transduction where Rac involves. Moreover, the RAFFU ring of lame RIPODIA (lobopodium) of a cell or a cell membrane which is the physiological function of Rac may be guided, and the intervention to cell adhesion is presumed.

[0010] about the intracellular localization of an APC gene product, in case a cell moves to a large

intestine villus tip from KURI[redacted]TO, piling up at least the microtubule p[redacted]f the cell which moves is reported -- **** (179 J. Cell Biol., 134:165- 1996) -- it found out also accumulating the new protein M1 of this invention on the same part. From this, the new protein M1 of this invention may have grasped the key of the cell migration control in a large intestine villus.

[0011] (Polypeptide) The new protein M1 of this invention is a polypeptide which consists of an amino acid sequence shown in the array number 1 of an array table. Furthermore, the polypeptide of this invention is chosen from the polypeptide which has the partial array of the polypeptide shown in the array number 1 of this array table. The polypeptide chosen has more preferably the homology who surpasses about 90% still more preferably about 80% or more about 70% or more as preferably as the polypeptide shown in the array number 1 of an array table. Selection of a polypeptide with this homology can be performed by making affinity with the armadillo repeat domain of for example, an APC gene product into an index.

[0012] the technique of determining the homology of an amino acid sequence -- the very thing -- it is well-known, for example, the approach of determining an amino acid sequence directly, the method of presuming the amino acid sequence by which a code is carried out to this after determining the base sequence of the polynucleotide presumed, etc. can be used.

[0013] The polypeptide of this invention can use the amino acid sequence chosen from the polypeptide which has the partial array of the polypeptide which consists of an amino acid sequence shown in the array number 1 of an array table as a reagent, the standard substance, and immunogen. As the smallest unit, at least preferably, it consists of an amino acid sequence which consists of about 11-15 or more amino acid at least still more preferably, and about five or more polypeptides [about 8-10 or more] which can be screened immunologically are set as the object of this invention at least.

[0014] Furthermore, the polypeptide which consists of an amino acid sequence which has variation or induced mutation, such as 1 thru/or deletion, a permutation, addition of some amino acid, can also be offered by making affinity with the armadillo repeat domain of an APC gene product into an index based on the polypeptide specified in this way. the means of deletion, a permutation and addition, or insertion -- the very thing -- it is well-known, for example, the technique (219:666 Science, 1983) of Ulmer can be used. Furthermore, these peptides that can be used are changeable to extent without remarkable modification of functions, such as embellishing the configuration amino group or carboxyl group etc.

[0015] The polypeptides of this invention are these very thing, and can be used for the physic constituent for adjusting the function of the new protein M1. Moreover, the polypeptide of this invention can be used for the screening for obtaining the compound which can adjust the function of the new protein M1, for example, an inhibitor, an antagonist, an activator, etc., and acquisition of the antibody to the new protein M1. Furthermore, the polypeptide of this invention is usable also as a reagent and a reference standard.

[0016] (Polynucleotide) The polynucleotide in which the peptide which has at least 15 continuous base sequences, and carries out a code has a binding affinity with the arm domain of an APC gene product is meant among the complementary strand to the polynucleotide and this polynucleotide of the array number 2 of an array table to which the polynucleotide of this invention and its complementary strand carry out the code of the amino acid sequence given in the array number 1 of an array table, these polynucleotides and the polynucleotides which carry out hybridization under stringent conditions, and these polynucleotides. If DNA is taken for the example of representation as a polynucleotide, "DNA hybridized under stringent conditions to DNA" can be obtained by the approach of a publication to above-mentioned Moleculer Cloning. In the inside of the solution of 0.1xSSC and 0.5%SDS after warming at 42 degrees C here in the solution of for example, 6xSSC, 0.5%SDS, and 50% formamide, saying "high BURITAIZU is carried out under stringent conditions" It means that the signal of electropositive high BURITAIZU is still observed also on the conditions washed at 68 degrees C.

[0017] The polynucleotide of this invention means the homologous chain and complementary strand which carry out the code of the amino acid sequence of a publication to the array number 1 of an array table and which are chosen from the information on the polynucleotide of the array number 2 of an array

table, and means the polynucleotide array which consists of about 15-20 or more arrays at least and this complementary strand corresponding to a field of the specified nucleotide sequence. Manifestation protein can be simply checked using this protein manifestation system with the well-known decision of a useful polynucleotide array, for example, an acellular protein manifestation system, and it can carry out by making that bioactive, especially affinity with the armadillo repeat domain of an APC gene product into an index, and sorting out them. As an acellular protein manifestation system, the technique of the ribosome system of the origins, such as a germ and rabbit reticulocyte, can be used, for example (161 160- Nature, 179, 1957).

[0018] Each of these polynucleotides can provide manufacture of the new protein M1 of this invention, and the polypeptide of this invention with useful gene information, and it can be used for them as the probe or primer for nucleic acids, such as a gene which carries out the code of these, or mRNA detection, or antisense oligomer for adjusting gene expression. For example, when using the polynucleotide of this invention as antisense, the manifestation of M1 is specifically checked by using the nucleotide sequence of a field peculiar to other known protein M1, for example, new protein other than a consensus sequence field with the KIAA0424 grade which is one of the Dbl families. Furthermore, the polynucleotide of this invention can be used also as the reagent and a reference standard about a nucleic acid.

[0019] (Transformant) except for the above acellular protein manifestation systems -- the very thing, such as Escherichia coli, yeast, a Bacillus subtilis, an insect cell, and an animal cell, -- the polypeptide which consists of new protein M1 which consists of this invention, and its origin object with the gene modification technology using a well-known host can be offered. In the example of this invention, although COS-7 cell was used, of course, it is not limited to this.

[0020] a transformation -- the very thing -- a well-known means can be applied, for example, a host's transformation is performed as replicon using a plasmid, a chromosome, a virus, etc. Although the method of integrating into a chromosome will be raised as a more desirable system if the stability of a gene is taken into consideration, the autonomous replication system using an extranuclear gene is used simple. A vector is chosen by host's class and uses as a component the gene sequence which supported the information about the gene sequence, the duplicate, and control for the purpose of a manifestation. a component -- a host -- a prokaryotic cell or an eukaryotic cell -- choosing -- about a promotor and a ribosome bond part -- a terminator, a signal sequence, an enhancer, etc. -- the very thing -- it is combined and used by the well-known approach.

[0021] a transformant -- the very thing -- it can use for manufacture of the polypeptide of this invention by choosing and cultivating the optimal conditions for each well-known host's culture condition. Although the bioactive of the polypeptide which consists of new protein M1 by which manifestation production is carried out, and its origin object, especially affinity with the armadillo repeat domain of an APC gene product may be made into an index and culture may perform them, it makes an index the amount of transformants in a culture medium, and subculture or a batch performs it.

[0022] (Recovery of the new protein M1 and its origin object) Recovery of the polypeptide which consists of the new protein M1 and its origin object from a culture medium makes an index affinity with the armadillo repeat domain of an APC gene product, combines molecular sieving, an ion column chromatography, an affinity chromatography, etc., or can carry out purification recovery also with fractionation means based on a solubility difference, such as an ammonium sulfate and alcohol. More preferably, based on the information on an amino acid sequence, the antibody to this amino acid sequence is created, and the approach of carrying out adsorption recovery specifically by the polyclonal antibody or the monoclonal antibody is used.

[0023] (Antibody) An antibody sorts out and creates the antigenic determinant of the polypeptide which consists of the new protein M1 and its origin object of this invention. At least five antigenic determinants consist of at least 8-10 amino acid more preferably. This amino acid sequence does not necessarily need to be the array number 1 and homologous of an array table, and if an exposed part is only discontinuity, it is [that what is necessary is just an exposed part to the exterior on a proteinic spacial configuration] effective [an amino acid sequence] that it is also an amino acid sequence

continuous about this exposed part. In the example, the fragment of the pl[redacted] 73rd] - the 126th place of an amino acid sequence was used as immunogen. An antibody is not limited especially as long as the polypeptide which consists of new protein M1 and its origin object immunologically is recognized. A well-known antigen antibody ligation reaction determines the existence of this recognition.
[0024] In order to produce an antibody, the polypeptide which consists of the new protein M1 and its origin object of this invention is combined with independent or support under existence of an adjuvant or nonexistence, and immunity induction of a humoral response, a cellularity response, etc. is performed to an animal. Support is not limited especially if self does not cause adverse reaction to a host, for example, a cellulose, a polymerized amino acid, albumin, etc. are illustrated. As an animal which carries out immunity, a mouse, a rat, a rabbit, a goat, a horse, etc. are used suitably. a polyclonal antibody -- the very thing -- it acquires by the antibody collecting method from a well-known blood serum. As a desirable means, it is the immunity affinity chromatography method. In the example, the affinity chromatography which combined GST-M1 refined anti-M1 antibody.
[0025] the animal to which the above-mentioned immunity means was given in order to produce a monoclonal antibody to an antibody forming cell -- collecting -- the very thing -- it is carried out by introducing the transformation means to a well-known permanent clonogen.
[0026] A polyclonal antibody or a monoclonal antibody combines with the new protein M1 which consists of direct this invention, is controllable in the activity and can control the interaction system of an APC gene product and the new protein M1 easily. Therefore, it is useful because of the therapy and prevention of the disease to which an APC gene product and the new protein M1 relate.
[0027] The polypeptide which consists of new protein M1 prepared in this way and its origin object, (Screening) The cell to which the transformation of these was carried out based on the information on the polynucleotide which carries out a code and its complementary strand, these amino acid sequences, and a base sequence, The antibody which recognizes immunologically the polypeptide which becomes a list from the new protein M1 and its origin object Independent or the affinity of the polypeptide and APC gene product which consist of new protein M1 and its origin object by combining two or more means, A means effective in screening of the inhibitor or activator to the manifestation of functions, such as the GEF activity of the new protein M1, or the new protein M1 is offered. By namely, the thing of the polypeptide of this invention, and the antibody of this invention for which any one is used at least The screening approach for obtaining the compound which checks or reinforces the affinity of the polypeptide of this invention and an APC gene product The polynucleotide of this invention, the vector of this invention, the transformant of this invention, The screening approach of the compound which interacts with the polynucleotide of this invention, and checks or reinforces the manifestation of this polynucleotide by the thing of the antibody of this invention for which any one is used at least The screening approach of the compound which checks or reinforces functions, such as the GEF activity of the polypeptide of this invention, can be offered by the thing of the polypeptide of this invention, and the antibody of this invention for which any one is used at least. for example, sorting of the antagonist by the drug design based on the spacial configuration of a polypeptide, sorting of the manifestation regulator in the gene level using a protein manifestation system, sorting using an antibody of the antibody recognition matter, etc. -- the very thing -- in a well-known drugs screening system, it is available.
[0028] (A compound, physic constituent) The compound obtained by the above-mentioned screening approach is available as candidate compounds, such as the inhibitor and antagonist which adjust functions, such as an interaction of the polypeptide and APC gene product which consist of new protein M1 and its origin object, or the GEF activity of the new protein M1, and an activator. Moreover, it is available also as candidate compounds, such as the inhibitor and antagonist to the manifestation of the polypeptide which consists of the new protein M1 and its origin object in gene level, and an activator. As candidate compounds, such as the above-mentioned inhibitor, an antagonist, and an activator, protein, a polypeptide, the polypeptide that does not have antigenic, a low molecular weight compound, etc. are mentioned, and it is a low molecular weight compound preferably.
[0029] The candidate compound sorted out in this way can be prepared as a physic constituent used for

the therapy of a large intestine neoplasm by sorting out in consideration of balance of biological usefulness and toxicity. Moreover, these very thing can use the antibody which recognizes immunologically the polypeptide which becomes a vector list including the polypeptide which consists of new protein M1 which consists of this invention, and its origin object, the polynucleotide which carries out the code of these and its complementary strand, and these base sequences from the new protein M1 and its origin object as a physic means used for the therapy of the large intestine neoplasm which has functions, such as inhibition, antagonism, activation, etc. to the interaction of the new protein M1 and an APC gene product Here, specifically with a large intestine neoplasm, the familial adenoma nature polyposis (FAP) and colon cancer are mentioned including a benign tumor and a malignant tumor. in addition -- if in charge of pharmaceutical preparation-ization -- the very thing -- a well-known polypeptide, protein, a polynucleotide, an antibody, etc. should just introduce the pharmaceutical preparation-ized means according to each set elephant.

[0030] The polypeptide which consists of new protein M1 which consists of this invention, and its origin object, In a vector list including the polynucleotide which carries out the code of these and its complementary strand, and these base sequences The antibody which recognizes immunologically the polypeptide which consists of new protein M1 and its origin object It can be used as diagnostic means, such as a disease relevant to the manifestation of the disease M1 to which the manifestation of the polypeptide of this invention or its activity relates, for example, the new protein of this invention, or an interaction with an APC gene product. Especially, it is useful as diagnostic means, such as a diagnostic marker of a large intestine neoplasm, and/or a reagent. A diagnosis is performed determining the abundance of the ****ing nucleic-acid array using an interaction and reactivity with the nucleic-acid array which is carrying out the code of the new protein M1, determining biodistribution about the new protein M1, and/or by determining the abundance in the inside of the sample of the new protein M1. In detail, the new protein M1 is authorized as a diagnostic marker. the measuring method -- the very thing -- what is necessary is just to use a well-known antigen-antibody reaction system, an enzyme reaction system, the PCR system of reaction, etc. In addition, the means said here means the approach and/or medium which are used for the purpose achievement. That is, for example, the approach for diagnosing, the reagent kit used for a diagnosis are contained in a diagnostic means.

[0031]

[Example] Hereafter, although this invention is concretely explained based on an example, this invention is not limited to the following example.

(Cloning [of cDNA]) 2 hybrid screening (two hybrid screens) (CLONTECH MATCHMAKERTM Two-Hybrid System) was performed for the purpose of obtaining the protein which has a binding affinity to the armadillo repeat domain (it may be hereafter called APC-arm for short) of an APC gene product. Namely, GAL4 with which the Homo sapiens APC gene product armadillo repeat domain (amino acid residue 446-880) was united as Bate (bait) It screened for the Homo sapiens embryo brain (human fetal brain) cDNA library (Clontech) using plasmid GBT9-APC containing a DNA binding domain. Transfection of this cDNA library and Bate was carried out to the yeast which introduced his3 and lacZ as a reporter gene, and the electropositive clone was detected by making beta-gal assay and HIS auxotroph into an index.

[0032] One electropositive clone was obtained from 1.1x107 transformants. It was a new array when DNA sequence determined the base sequence of the cDNA fragment which carries out the code of the polypeptide combined with APC-arm discovered by this clone from this obtained clone. The array of the downstream region of the obtained cDNA fragment is 'Marathon'-ready. It acquired by the 3'RACE system (Clontech) using the Homo sapiens brain cDNA (Clontech). The primer used 5'-CGACATCTGCGAGGGCTACGTCCGG-3'. Moreover, it screened by the hybridization which used the digoxigenin (digoxigenin;DIG) indicator probe about the human genome library (Clontech) by using as a probe a part of array (an array table, base number 97-269 of the array number 2) of the cDNA fragment obtained above. Consequently, two clones containing the base number 1-81 of an array table and the array number 2 to overlap were obtained, and the array of the perfect length cDNA of the protein combined with target APC-arm was determined.

[0033] (Amino acid sequence) cDNA shown in the array number 2 of an array table obtained by the above-mentioned approach had the new base sequence. Based on this cDNA, the presumed amino acid sequence of the new protein M1, i.e., the array of the amino acid residue 619 shown in the array number 1 of an array table, was acquired by the translation of the base sequence (drawing 1 A).
[0034] (Homology with the existing protein) Using the presumed amino acid sequence of this new protein M1, when homology retrieval using BLAST (The National Centerfor Biotechnology Information) was performed to the existing database (Genbank), KIAA0424 and 73% of homology who are one of the subfamily members of a Dbl family were accepted (drawing 2 B and C). Although both were the same in the point of holding a Dbl homologous (Dbl homology;DH) domain, a PUREKKUSUTORIN homologous (Preckstrin homology;PH) domain, and Src homologous 3 (SH3) domain, to KIAA0424, it became clear that the N terminal region of the new protein M1 did not exist. Consequently, it was checked that the protein M1 of this invention which has the amino acid sequence shown in the array number 1 of an array table is new protein.
[0035] (Check of a manifestation organization) The manifestation in the human tissue of the new protein M1 shown below according to the presumed amino acid sequence of the array number 1 of an array table was checked by NOZAN blotting analysis. a variety -- human tissue -- from -- having obtained -- poly -- (-- A --) -- + -- RNA -- a blot -- having carried out -- a filter -- Clontech -- a shrine -- receiving -- DIG -- an indicator -- having carried out -- M -- one -- cDNA -- a probe -- five -- ' - GACCACACTGCCATCGCTG - three -- ' -- and -- five -- ' - TGTAGTTTACCAAGGACCG - three -- ' -- hybridization -- having carried out . Consequently, it checked that it was discovered with a high level in the brain. Moreover, tissue In the analysis by blots, existence was checked also by the testis in addition to the brain. Furthermore, the manifestation was checked also in the cell of the kidney origin.
[0036] (Production of an antibody) Using the peptide containing the amino acid 73-126 of M1, by the well-known approach, immunity of the NZW rabbit was carried out and the antibody to M1 prepared it. Using the peptide containing the amino acid 1122-1729 of an APC gene product (it may call for short Following APC), by the well-known approach, immunity of the NZW rabbit was carried out and the antibody to APC prepared it. The mouse monoclonal antibody to the N terminal region of APC was prepared by the well-known approach (Miyashiro et al.1995). The antibody was refined by performing affinity chromatography using the affinity column which combined the antigen used for immunity, respectively. When the joint reactivity of rabbit polyclonal anti-M1 refined antibody and the refined GST fusion M1 (GST-M1) was investigated, the obtained antibody showed reactivity strong against GST-M1.
[0037] (Manifestation of M1) In order to make the new protein M1 of this invention discover, cDNA of M1 was included in the EcoRI/NotI part of the manifestation plasmid pcDNA3.1 (+), and transfection was carried out to COS-7 cell. Transfection of the vector incorporating M1cDNA, a control vector, the vector incorporating M1cDNA which carried out the indicator with HA-tag, and the vector incorporating HA-tag was carried out to COS-7 cell for the manifestation check of the new protein M1. Immunoprecipitation was carried out using anti-M1 antibody acquired as mentioned above and anti-HA antibody about the melt of each cultivated transformant, and the melt of a rat embryo brain by which existence of M1 is checked, fractionation was carried out by SDS-PAGE, and, subsequently the immuno blot was performed using anti-M1 antibody and anti-HA antibody. In COS-7 cell and rat embryo brain (lane 1) which carried out the transformation by the vector (lane 3) incorporating M1cDNA, and the vector (lanes 6 and 8) incorporating M1cDNA which carried out the indicator with HA-tag, the manifestation of new protein was accepted clearly and it became clear that the molecular weight was about 85 kDa(s) (drawing 2). moreover, anti-M1 used antibody -- an antigen -- beforehand -- processing (it displaying by pep.** among drawing 2) -- new protein was not detected. That is, it was checked that the new protein M1 recognized by anti-M1 antibody had been discovered.
[0038] (Analysis of association in in vivo of M1 and APC) About the rat embryo brain melt, immunoprecipitation was carried out using the rabbit polyclonal antibody to the new protein M1 and APC produced above, and the mouse monoclonal antibody (Transduction Laboratories) to beta-KATENIN, and SDS-PAGE performed the immuno blot after separating each sediment using each

antibody (drawing 3). In lanes 1, 3, and 5, lanes 1 and 2 show the result ([illegible]splays by pep.** among drawing 3) which absorbed the result to which it is anti-M1 antibody, lanes 3 and 4 are anti-APC antibodies, and lanes 5 and 6 carried out immunoprecipitation of the melt of a rat embryo brain by anti-beta-KATENIN antibody with the antigen which corresponds each antibody beforehand, and was used. The new protein M1 since beta-KATENIN which the APC gene product which M1 which carried out immunoprecipitation by anti-M1 antibody was detected by the anti-APC antibody or anti-beta-KATENIN antibody, and carried out immunoprecipitation by the anti-APC antibody was detected by anti-M1 antibody, and carried out immunoprecipitation by anti-beta-KATENIN antibody is detected by anti-M1 antibody is in. It became clear to have combined with APC and beta-KATENIN also in vivo.
[0039] (Analysis of the interaction part of M1 and APC) In order to analyze the APC joint domain of the new protein M1, various deletion mutants (deletion mutant) of M1 were produced by the well-known approach, and two hybrid systems using yeast (yeast) investigated the joint field with APC. Specifically PCR was performed using the following specific primer, cloning of the deletion mutant of M1 was carried out to pGAD424, and fusant with a GAL4 activation domain was created.
5 '-ATTTATTGTAGTTTACCAAGGAC-3'5'-TGCGCTGAAGCACTCTGGGAC-3' 5'-GACCACACTGCCATCGCTGATG-3'5'-CCTCAGCCGAACGAAGCTGGCTG-3'5'-CTTGCTGC TCTGCGCCTCCGC- 3'5' -GTGAATCAGGACGAGCCCGCG-3'5 -- '-GATGTTCCCGAAGATGGTACG-3'5'-ATGCCTGATGGAGCTCTGGAC-3' -- subsequently Fusant with this GAL4 activation domain is set to two hybrid systems, and it is HIS3. auxotrophy and beta-gal The interaction was examined using reporter activity. Consequently, as shown in drawing 4 , it became clear that an APC binding site existed in the field of the upstream of SH3.
[0040] (Joint analysis of M1 and Rho family low-molecular-weight G protein) The new protein M1 has KIAA0424 which is one of the subfamilies of a Dbl family, and high homology. A Dbl family is GDP dissociation promotion protein which acts on the Rho family which is one of the low-molecular-weight G protein. Paying attention to homology with the new protein M1 and KIAA0424, association with Rho family low-molecular-weight G protein (small G protein;RhoA, Rac1, CDC42) was investigated for the functional analysis of the new protein M1. The new protein M1 made to stick to a nickel bead RhoA, Rac1, and GST-CDC42, E1A buffer-solution [50mM HEPES containing 0.1%NP-40, pH7.0,150mMNaCl, 50mM NaF, 5mM EDTA, 1mMDTT, 50microg/mL phenylmethylsulphonyl fluoride (PMSF), 1microg/ml It mixed at 4 degrees C in leupeptin and 1microg [/ml] aprotinin] for 1 hour, and the coprecipitation Mr. object was detected by immunoblotting. As shown in drawing 5 , Dbl used as electropositive control combined with all the used Rho family low-molecular-weight G protein, but the new protein M1 did not combine CDC42, although RhoA and Rac1 were combined.
[0041] (GEF activity of M1) Next, the GEF activity of the new protein M1 was examined. The low-molecular-weight G protein of the [3H] GDP joint mold used in order to investigate GDP dissociation from low-molecular-weight G protein was obtained by incubating each low-molecular-weight G protein of 2pmol at 0.2microM[3H] GDP and 30 degrees C in 20 minutes and in the buffer solution for installation (loading buffer;20mM Tris-HCl, pH8.0, 3mM MgCl2, 10mM EDTA, and 1mM dithiothreitol). It is 375mM in order to prevent dissociation of [3H] GDP from low-molecular-weight G protein. In addition, this mixed liquor was immediately ice-cooled so that final concentration might serve as 18mM(s) in MgCl2. [3H] Dissociation of GDP was performed at 25 degrees C by adding the non-indicators GDP, GTP, and M1 with 250 superfluous times to a reaction solution (20mM Tris-HCl, pH8.0, 6mM MgCl2, 3.5mM EDTA, and 1mM dithiothreitol) (drawing 6 A and B). Next, each low-molecular-weight G protein of 2pmol is incubated in 20 minutes and in the buffer solution for installation (loading buffer) at the 0.2microM non-indicator GDP and 30 degrees C in the low-molecular-weight G protein of the GDP joint mold used in order to investigate association of GTP to low-molecular-weight G protein, and it is 375mM. It obtained by adding MgCl2 so that final concentration may serve as 18mM(s). [35S] Association to the GDP joint mold low-molecular-weight G protein of GTPgammaS was carried out at 25 degrees C by adding [35S] GTPgammaS of 10microM, and M1 to a reaction solution (drawing 7 A and B). both a dissociation test and a joint trial -- setting -- a reaction -- the ice-cooled 1ml buffer solution for a halt (20mM Tris-HCl, pH8.0, 25mM MgCl2, and

100mM(s) NaCl) -- in addition, it stopped. The diluted mixed solution was filtered through the nitrocellulose filter paper, and the buffer solution for a halt washed the filter several times. The radioactivity caught on the filter paper was counted. Protein concentration was measured using bovine serum albumin (BSA) as standard protein.

[0042] As shown in drawing 6 , the new protein M1 acted on Rac1 which is the low-molecular-weight G protein which can be combined, and GDP dissociation from Rac1 was promoted, but (drawing 6 A) since it did not combine with RhoA, it did not act on GDP dissociation from RhoA (drawing 6 B). moreover, it is shown in drawing 7 -- as -- the new protein M1 -- GTP association to Rac1 -- having promoted (drawing 7 A) -- GTP association to RhoA was not influenced (drawing 7 B). That is, the new protein M1 of this invention acts on low-molecular-weight G protein, and has GEF activity.

[0043] (Operation of APC over the GEF activity of M1) Under existence of APC-arm or nonexistence, at 30 degrees C, the incubation of the [3H] GDP dissociation promotion ability from Rac1 of a joint mold which combined 20[3H] of nM GDP was carried out for 15 minutes, and it was measured about M1 of still more various concentration. APC-arm was added so that it might become 5 times from M1 by the excessive amount and the mole ratio. As shown in drawing 8 , the GDP dissociation promotion ability of M1 was reinforced by addition of APC-arm. That is, when M1 combined with APC, it was suggested that GDP dissociation promotion ability is reinforced.

[0044] (Intracellular localization of M1) Next, the intracellular localization of the new protein M1 of this invention was examined. First, Madin-Darby canine Dulbecco's which contains fetal calf serum (FBS) for a kidney (MDCK) epithelial cell stock 10% modified Eagle's It cultivated in medium (DMEM). M1 and M1**NB(amino acid 127-619) cDNA of an overall length carried out sub cloning into the mammalian expression vector pcDNA3.1 (+) which has a CMV promotor. APC-arm which carried out the indicator with plasmid DNA and a pMKITNeo-Myc tag was built by inserting the DNA fragment which is carrying out the code of the partial array of APC (amino acid 446-880) in pMKITNeo which has SRalpha promotor. The manifestation plasmid transfected the MDCK cell according to the user manual using lipofectamine (LipofectAMINE;Life Technologies).

[0045] The MDCK cell was fixed at 4 degrees C for 1 hour using formaldehyde 3.7% in phosphate buffered saline (Phosphate Buffered Saline;PBS). The fixed cell was processed with the tris buffered saline solution (Tris Buffered Saline;TBS) which contains a triton X-100 0.2% for 10 minutes at a room temperature, and was washed 3 times in TBS. After transparency-izing a cell (permeabilize), it incubated at the primary antibody and the room temperature 3% for 1 hour all over 1%BSA, and FBS and TBS which contains a triton X-100 0.2%. The primary antibody was removed and the cell was washed 3 times in TBS. The united primary antibody was detected using the goat second antibody (Cappel) which combined FITC (Fluorescein isothiocyanate) or a rhodamine. The dyed sample was observed under the KARUTSU ice LSM510 laser scanning microscope (Carl Zeiss LSM510Laser scanning microscope).

[0046] A result is shown in drawing 9 . The same cell which made M1 protein which carried out the indicator of a and the b with HA-tag among drawing 9 discover, The same cell which made both of APC-arm which carried out the indicator with M1 protein which carried out the indicator of c and the d with HA-tag, and a Myc-tag discover, The cell which made M1**NB (amino acid 127-619) which carried out the indicator of the e with HA-tag discover, and f are the cells which made APC-arm which carried out the indicator with the Myc-tag discover. Moreover, the double stain of a and the b was carried out by anti-HA antibody and the anti-APC antibody, the double stain of c and the d was carried out by anti-HA antibody and the anti-Myc antibody, e is anti-HA antibody and f was processed by the anti-Myc antibody. As the arrow-head head in b showed, it existed in the film which the cluster of APC protein is elongating and the arrow head in a-d showed, it became clear that M1 and APC lived together to the same part of the verge of a cell in an epithelial cell.

[0047] (Gestalt analysis of M1 manifestation cell strain) The MDCK cell strain which discovers M1**NB to stability was established further, and when the effect which M1 has on the gestalt of a cell and a frame was investigated, the inclination which pastes up densely no M1**NB manifestation cell strain 5 clones, but is increased scatteringly was seen. The intervention to the cell adhesion of the new

protein M1 was presumed.

[0048]

[Effect of the Invention] As explained above, M1 of this invention is new protein, and affinity with the armadillo repeat part of the APC gene product characterizes it, and it has GEF (guanine-nucleotide exchange factor: Guanine nucleotide Exchange Factor) activity. Offer of the new physic constituent and the medical-examination means which this property was used offers big usefulness in the medical field of an APC gene product-related clinical and a foundation.

[Layout Table]

Sequence Listing<110> Daiichi Pharmaceutical Co.,Ltd.<120> Colon carcinoma suppressor gene related protein<130> DP99-1045<160> 2<210> 1<211> 619<212> PRT<213> human<400> 1Met Arg Pro Asp Gly Gln Gln Ala Leu Asp Ala Val Val Arg-Ser-Phe 1 5 10 15 Asp CysHis Ser Glu Ala Ala LeuArg Gln Arg Asn Asp Val Ile Tyr 20 25 30 Cys Ser Leu Pro Arg Thr Ala Gln Gly Ile Val Gln Arg Glu Asp Gln 35 40 45 Leu Glu Val Leu Val Ser Leu Arg Glu Val Trp Gly Arg Arg Arg Gly 50 5560 Arg Asp Gly Thr Cys Thr Gly Ala Met ProAsp Gly Ala Leu Asp Thr 65 70 75 80 Ala Val Cys Ala AspGlu Val Gly Ser Glu Glu Asp Leu Tyr Asp Asp 85 90 95 Leu His Ser Ser Ser His His Tyr Ser His Pro Gly Gly Gly Gly Glu100 105 110 Gln Leu Ala Ile Asn Glu Leu Ile Ser Asp Gly Ser Val Val Cys Ala 115 120125 Glu Ala Leu Trp Asp His Val Thr Met Asp Asp Gln Glu Leu Gly Phe 130 135 140 Lys Ala Gly Asp Val Ile Glu Val Met Asp Ala Thr Asn Arg Glu Trp 145 150 155 160 Trp Trp Gly Arg Val AlaAsp Gly Glu Gly Trp Phe Pro Ala Ser Phe 165 170 175 Val Arg Leu Arg Val Asn Gln Asp Glu Pro Ala Asp Asp Asp Ala Pro 180 185190 Leu AlaGly Asn Ser Gly Ala Glu Asp Gly Gly Ala Glu Ala Gln Ser 195 200 205 SerLys Asp Gln Met Arg Thr Asn Val Ile Asn Glu Ile Leu Ser Thr 210 215 220 Glu ArgAsp Tyr Ile Lys His Leu Arg Asp Ile Cys Glu Gly Tyr Val 225 230 235 240 Arg Gln Cys Arg Lys Arg Ala Asp Met Phe Ser Glu Glu Gln Leu Arg 245 250 255 Thr-Ile-Phe-Gly-Asn Ile Glu Asp Ile Tyr-Arg-Cys-Gln-Lys-Ala-Phe 260 265 270 Val Lys Ala Leu Glu Gln Arg Phe Asn Arg Glu Arg Pro His Leu Ser 275280 285 Glu Leu Gly Ala Cys Phe Leu Glu His Gln Ala Asp Phe Gln Ile Tyr 290 295 300 SerGlu Tyr Cys Asn Asn His Pro Asn Ala Cys Val Glu Leu Ser Arg 305 310 315 320 Leu Thr Lys Leu Ser Lys Tyr Val Tyr Phe Phe Glu Ala Cys Arg Leu 325 330335 Leu Gln Lys Met Ile Asp Ile Ser Leu Asp Gly Phe Leu Leu Thr Pro 340 345350 ValGln Lys Ile Cys Lys Tyr Pro Leu Gln Leu Ala Glu Leu Leu Lys 355 360 365 Tyr Thr His Pro Gln His Arg Asp Phe Lys Asp Val Glu Ala Ala Leu 370 375 380 His Ala Met Lys Asn Val Ala Gln Leu Ile Asn Glu Arg Lys Arg Arg 385 390 395400 LeuGlu Asn Ile Asp Lys Ile Ala Gln Trp Gln Ser Ser Ile Glu Asp 405 410 415 Trp Glu Gly Glu Asp Leu Leu Val Arg Ser Ser Glu Leu Ile Tyr Ser 420 425 430 Gly Glu Leu Thr Arg Val Thr Gln Pro Gln Ala Lys Ser Gln Gln Arg 435 440 445 Met Phe Phe Leu Phe Asp His Gln Leu Ile Tyr Cys Lys Lys Asp Leu 450 455 460 LeuArg Arg Asp Val Leu Tyr Tyr Lys Gly Arg Leu Asp Met Asp Gly 465 470 475480 Leu Glu Val Val AspLeuGlu AspGly Lys Asp Arg Asp Leu His Val 485 490 495 Ser Ile Lys Asn Ala Phe Arg Leu His Arg Gly Ala Thr Gly Asp Ser 500 505 510 His Leu Leu Cys Thr Arg Lys Pro Glu Gln Lys Gln Arg Trp Leu Lys 515 520 525 Ala-Phe-Ala-Arg-Glu Arg Glu Gln Val Gln-Leu-Asp-Gln-Glu-Thr-Gly 530 535 540 Phe Ser Ile Thr Glu Leu Gln Arg Lys Gln-Ala Met Leu Asn Ala Ser 545 550555 560 Lys Gln Gln Val Thr Gly Lys Pro Lys Ala Val Gly Arg Pro Cys Tyr 565570 575 Leu Thr Arg Gln Lys His Pro Ala Leu Pro Ser Asn Arg Pro Gln Gln 580 585 590 Gln Val Leu Val Leu AlaGluPro Arg Arg Lys Pro Ser Thr Phe Trp 595 600 605 His Ser Ile Ser Arg Leu Ala Pro Phe Arg Lys * 610 615 620 <210> 2 <211> 1860 <212> cDNA <213> human <400> 2 atg agg cca gat ggg cag caa gct ttg gat gct gtg gta agg agt ttt 48 Met Arg Pro Asp Gly Gln Gln Ala Leu Asp Ala Val Val Arg Ser Phe 1 5 10 15 gac tgt cac tct gaa gcagct ctc agg cag agg aat gat gtg att tat 96 Asp Cys His Ser Glu Ala Ala Leu Arg Gln Arg Asn Asp Val Ile Tyr 20 25 30 tgt agt tta cca agg acc gct cag ggt att gtg cag agg gaa gac cag 144 Cys Ser Leu Pro Arg Thr Ala Gln Gly Ile Val Gln Arg Glu AspGln 35 40 45 ctg gag gttctt gtg tca ctc cgt gaa gtg tgg ggg cggcgg agg ggc 192 Leu Glu Val Leu Val Ser Leu Arg Glu Val Trp Gly Arg Arg Arg Gly 50 55 60 aga gat ggg acc tgc act gga gcc atg cct gatgga gct ctg gac aca 240 Arg Asp Gly Thr Cys Thr Gly Ala Met ProAsp Gly Ala Leu Asp Thr 65 70 75 80 gct gtc tgc gct gac gaa gtg ggg agc gag gag gac ctg tat gat gac 288 Ala Val Cys Ala Asp Glu Val Gly Ser Glu Glu AspLeu Tyr Asp Asp 85

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